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JANUARY, 1929

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Archives of Internal Medicine

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ANEURYSM OF THE CORONARY ARTERIES*

MAURICE PACKARD, M.D.
AND
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NEW YORK

Although the coronary arteries are so frequently involved in the general diseases of the vascular system, aneurysm is an extreme rarity. In the 116 years that have elapsed since Bougon [1] (1812) reported the first case, after a careful search of the literature we have been able to collect only thirty-one examples of this condition—and at least two of these are extremely doubtful. This total does not include cases of periarteritis nodosa, a disease entity first described by Kussmaul and Maier [2] in 1866. The multiple beadlike aneurysms of the coronary arteries, which are frequently present in this disease, are only part of a widespread involvement of the medium sized vessels throughout the body and are best considered from that standpoint.

Few attempts have been made to collect the scattered case reports and to analyze them critically. The most elaborate of these are: Crisp [3] (1871), eleven cases; Capps [4] (1899), nineteen cases, and Griffith [5] (1901), twenty-four cases. Their tables, however, are incomplete and contain data for many cases in which there were no aneurysm of the coronary artery. For the sake of future workers in this field, it might be well to point out these errors. In the cases of Ward [6] and Stevenson [7] and in the specimen at St. Bartholomew's Museum [8] the aneurysms were of the sinus of Valsalva. That of Bristowe [8] and the one listed in the St. Thomas' Museum Catalogue and reported by Powell [9] are cases of periarteritis nodosa. In the case of Clark [10] the rupture of a coronary artery was not the seat of an aneurysm, and Shrady's [11] report

* Submitted for publication, Aug. 16, 1928.

* From the Medical Service of the Gouverneur Hospital.

1. Bougon: Biblioth. méd. **37**:183, 1812.
2. Kussmaul and Maier: Deutsche. Arch. f. klin. Med. **1**:484, 1865-1866.
3. Crisp: Tr. Path. Soc. London **22**:106, 1871.
4. Capps: J. A. M. Sc. **118**:312, 1899.
5. Griffith: Brit. M. J., 1901, p. 266.
6. Ward: Tr. Path. Soc. London **8**:134, 1857.
7. Stevenson, quoted by Crisp (footnote 3), p. 33.
8. Bristowe: Tr. Path. Soc. London **7**:98, 1856.
9. Powell, Reynolds: System of Medicine, New York, The Macmillan Company, 1879, vol. 5, p. 133.
10. Clark: New York M. J. **8**:384, 1860.
11. Shrady: New York M. Press **3**:225, 1860.

is merely an abstract of Clark's case. Wiegand [12] reported one case of aneurysmal dilatation of the ascending aorta, associated with coronary sclerosis. Griffith [5] referred to the report of a second case in the *St. Bartholomew Hospital Reports* for 1871, but we have been unable to discover any mention of it in that journal. He also listed two cases by Dr. Chadwick of Leeds, who "remembers having heard of them—one in the practice of one of his colleagues and the other in or about the hospital." For obvious reasons, these have not been included in our tables.

Eppinger,[13] who is not listed by either Capps or Griffith, described two cases, one of which is definitely periarteritis nodosa. He also mentioned a case recorded by Thompson.[14] The latter exhibited multiple aneurysms on the basis of an acute endocarditis, none of which, however, involved the coronary arteries. Baly's [15] case of thrombosis of the coronary artery was also excluded by Griffith.

Because of the meager descriptions of the heart and especially of the other organs of the body, and of the lack of adequate microscopic study in many of the cases reported, it has been impossible to decide on the exclusion of a few cases retained in our tables. The condition in two cases, particularly (Clarke [16] and Heuse [17]), is in all probability periarteritis nodosa. In addition, several other cases, such as those reported by Gee [18] and Malet and Evans,[19] are puzzling and unique. The data for two cases (Malmsten [20] and Herczel [21]) were unavailable.

ETIOLOGIC FACTORS

Sex.—Coronary aneurysm is almost three times as common in males as in females. In the twenty-five cases in which the sex was mentioned, eighteen were males and seven females.

Age.—Information concerning age is given in twenty-three cases in this series. The youngest patient was 5 and the oldest 77 years of age. One patient (Bougon) is described as being old. As can be seen from table 1, there is not a marked predilection for any decade.

Trauma.—In only three cases is indirect trauma considered as a possible etiologic factor. Peacock's [22] patient was a lifter of great

12. Wiegand: Med. Convers. Blatt **1**:217, 1830.
13. Eppinger: Arch. f. klin. Chir. **35**:1, 1887.
14. Thompson: M. Times & Gaz. **2**:56, 1877.
15. Baly: Pathol. Tr. **3**:264, 1850-1852.
16. Clarke: Pathol. Tr. **47**:24, 1896.
17. Heuse: Bull. Acad. roy. de méd. de Belg. **15**:492, 1856.
18. Gee: St. Bartholomew Hosp. Rep. **7**:148, 1871.
19. Malet and Evans: Lancet **2**:67, 1887.
20. Malmsten: Aorten-Aneurysmens Etiologie, Stockholm, 1888.
21. Herczel: Orvosi heti. szemle. **36**:149, 1904.
22. Peacock: Tr. Path. Soc. London **1**:227, 1846-1848; **9**:592, 1849.

weights; the patient in Crisp's [3] case suffered a prolonged submersion in water and a fall from a scaffold; while Fraenkel's [23] patient sustained wounds in the arm and head and severe shock.

Chronic Cardiovascular Disease.—Seven patients gave a history of rheumatic fever and one of rheumatic pains. Only three patients, including our own, exhibited a syphilitic meso-aortitis. One patient had had scarlatinal nephritis, one generalized arteriosclerosis and one cardiac disease.

SYMPTOMATOLOGY, PHYSICAL SIGNS AND DIAGNOSIS

There are not any typical symptoms referable to the aneurysm itself. In fact, in many cases there were apparently no complaints, or they were unknown at the time of the sudden death of the patient, either in the street or after such exertion as coitus.

Exclusive of a few unusual cases, the symptoms, when present, fall into two distinct groups: (1) There is a history of rheumatic fever

TABLE 1.—*Incidence of Aneurysm of the Coronaries According to the Age of the Patient*

Age	Number
0 to 10	2
10 to 20	3
20 to 30	4
30 to 40	5
40 to 50	2
50 to 60	1
60 to 70	4
70 to 80	2

with or without longstanding chronic valvular disease, and also the symptoms of an acute or subacute bacterial endocarditis; (2) the symptoms are those either of angina pectoris or of myocardial degeneration on a coronary basis.

In Ruge's [24] case, there was an acute osteomyelitis of the femur with pyemia, while in the case reported by Malet and Evans,[19] the only symptoms were thirst and polyuria. The patient in Capps' [4] second case had a hemiparesis, numbness and tingling in the feet and convulsions, probably due to a coincident syphilis of the central nervous system. Gee's [18] case, a child, aged 7, had a scarlatinal dropsy and died of an intercurrent pneumonia with meningitis.

Sudden death is common and is practically always due to rupture of the aneurysm. Gradual heart failure is the cause of death in the others.

Physical Observations.—There is nothing characteristic about the physical signs to differentiate the condition. They depend entirely on

23. Fraenkel: Deutsche. med. Wchnschr. **43**:159, 1917.
24. Ruge: Deutsche. Ztschr. f. Chir. **80**:150, 1905.

the underlying pathologic changes—acute and subacute bacterial endocarditis and coronary disease—and the degree of cardiac decompensation.

A pathognomonic sign has not been described in the few cases actually observed at the time of rupture.

Diagnosis.—From the foregoing, it is obvious that an antemortem diagnosis of coronary aneurysm is impossible, and it was not mentioned as a possibility in any of the cases in our series.

PATHOLOGIC OBSERVATIONS

The site of the aneurysm is stated in twenty-seven cases. The left coronary artery is the one most commonly affected, and in the vast majority, it is single. Multiple aneurysms were present in only six cases. The actual sites were as follows: left coronary, seventeen; right coronary, six; both coronaries, four. The bilateral cases include the doubtful ones of Heuse [17] and Clarke [16] and the unclassifiable one of Gee.[18] When the aneurysm is single, it is practically always situated immediately beyond the coronary orifice within the first inch of its course. The others, including the multiple aneurysms, are usually found at the points of division of the artery.

The aneurysms vary in size from that of a pea to that of a pigeon's egg. The largest was described by Martland [25] as being 6 cm. in diameter.

Rupture occurred in fourteen, or in nearly 50 per cent of the cases in our series. As described by Vestberg,[26] the eneurysm bursts first into the undefended or periaortic space, and then into the pericardial sac. In two cases (Sommer,[27] Windholz [28]), the perforation was into the conus arteriosus of the pulmonary artery.

Disregarding the few unique cases, the aneurysms themselves can be divided into two classes: (1) mycotic-embolic and (2) arteriosclerotic.

Mycotic-Embolic Aneurysm.—This term implies the synergistic action of two factors—the embolus itself and the mycosis or infection—and must be differentiated, on the one hand, from the pure mycotic aneurysm, of which we have an example in our series (Ruge), and on the other, from the pure embolic aneurysm, such as a calcareous plaque.[29]

Although as early as 1851, Koch [30] and, after him, several others,[31] described the association of an aneurysm of a terminal artery with an

25. Martland: Proc. New York Path. Soc. **17**:34, 1917.
26. Vestberg: Nord. med Ark. **7**:1, 1897.
27. Sommer: Frankfurt. Ztschr. f. Path. **5**:98, 1910.
28. Windholz: Centralbl. f. allg. Pathol. u. path. Anat. **37**:385, 1926.
29. Libman: Proc. New York Path. Soc. **5**:88, 1905.
30. Koch, L.: Inaug. Diss. Erlangen, 1851.
31. Tufnell: Dublin Quart. J. M. Sc. **15**:371, 1853. Waterman: West. M. J. **2**:584, 1867.

acute endocarditis of the aortic valve, Ponfick [32] is usually credited with being the first to recognize the connection between the endocardial vegetations and the peripheral aneurysms. He believed that they were caused by embolic calcified vegetations. In 1864, however, Ogle [33] definitely attributed the aneurysms present in his case to emboli from an aortic endocarditis. The inflammatory nature of the lesion was first established by Eppinger [13] in his exhaustive survey of the subject. Since that time, although many similar cases have been reported, they are not common, being only comparatively frequent in the subacute and chronic ulcerative forms of endocarditis. The incidence of aneurysm in the latter conditions can be gathered from the statistics of Lubarsch.[34] In 137 cases of subacute bacterial endocarditis and the recurrent chronic necrotizing form, he found embolic aneurysms in 10.2 per cent. The coronary arteries escaped entirely. In a large collection of cases, the superior mesenteric artery was the vessel most commonly affected.

In our series, there are seven cases (Bougon,[1] Ogle,[33] Eppinger,[13] Griffith [5] [cases 1 and 2], Henke,[35] Lublin [36]) which definitely belong in this category. Toller's [37] case probably belongs to this group, but unfortunately the condition of the heart valves is not mentioned. In three cases (Peacock,[22] Capps [4] [case 2], Windholz [28]), a healed chronic endocarditis was found, and the question naturally presents itself as to the possible etiologic significance of the acute stage of the process. After careful examination, we are inclined to place these cases in the arteriosclerotic group.

Of the seven unquestionable cases, only one was multiple and bilateral. The average age of the patients was 27. Rupture occurred in two cases (Ogle, Henke).

Depending on the stage of the process, the wall of the aneurysm is composed of varying amounts of relatively recent fibrous tissue, remnants of elastic fibers and more or less smooth muscle belonging to the media. At the entrance to the aneurysm, the intima and elastica cease abruptly and, at times, the ends of the latter have a curled appearance. Occasionally, a hyaline thrombus is found adherent to the floor. According to the completeness of the rupture, hemorrhage is present either in the adventitia or in the surrounding tissues. The wall is infiltrated with polymorphonuclear leukocytes and round cells. Plasma cells and endothelioid cells that contain blood pigment are often present. Streptococci or staphylococci are the usual causative bacteria.

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32. Ponfick: Virchows Arch. f. path. Anat. **58**:528, 1873.
33. Ogle: St. George's Hosp. Rep. **2**:285, 1867.
34. Henke and Lubarsch: Handb. d. spez. path. anat. u. histol. **2**:710, 1924.
35. Henke: Centralbl. f. allg. Pathol. u. path. Anat. **21**:1003, 1910.
36. Lublin: Centralbl. f. Herzkrankh. u. Gefässkrankh. **12**:171, 1920.
37. Toller: St. Thomas' Hosp. Rep., Lond. **31**:357, 1904.

TABLE 2.—*Report of Cases of Aneurysm in the Literature*

Author	Age	Sex	Occupation	Previous History	Symptoms and Physical Conditions	Site	Size	Rupture	Coronaries	Additional Pathologic Changes
1. Bougon, 1812...	Old	M	Soldier	Attacks of low fever and rheumatism	For four years, nocturnal anginal attacks; six weeks ago, fever and pain in calf of leg; sudden death	Right	.....	+	Right dilated to size of female sound	Vegetations on aortic valves contracting orifice to the size of a little finger
2. Merat (Dict. des se. méd. **5**: 484, 1813)	..	..	.....	.....	No cardiac symptoms.....	Left	Small nut	0		
3. Hedlund (A Otto's Compendium of Human and Comparative Anatomy. London, B. Fellowes, 1831, p. 319)	40	M	.....	....	.....	.....	.....	+		
4. Peste, 1843.....	77	M	Currier	Good health	Twenty-eight months ago, apoplexy with left hemiplegia; four days before death, malaise, vomiting, dyspnea and precordial pain; sudden death	Left	Large nut	+	Left dilated to the size of a brachial artery; calcified	Transverse rupture of the left ventricle; calcification of the aorta
5. Peacock, 1846...	51	M	Butcher	Irregular habits; lifter of weights; rheumatic pains	For months, severe cough and dyspnea; admitted in collapse ten days before death, with rapid feeble pulse, rapid respirations and cough; death by gradual cardiac failure	Left	Pigeon's egg	0	Dilatation and marked ossification of the left; right slightly dilated with scattered calcified plaques	Fibrinopurulent pericarditis; slight thickening and adherence of aortic cusps; several calcified plaques in the aorta
6. Heuse, 1856.....	21	M	Farmer	Three attacks of intermittent fever	For three months, symptoms of cardiac decompensation; hydrothorax; ascites; anasarca; sudden death	4 Both	5 to 9 mm. in diameter	+	Rupture of branches of coronaries in two places	Valves normal
7. Finnell (New York M. J. **16**: 82, 1856)	25	F	.....	....	Sudden death	Right	.....	0		
8. Markoe (New York M. J. **8**: 384, 1860)	..	F	.....	.....	Sudden death	.....	End of little finger	+		
9. Wood, 1860.....	..	M	.....	.....	Sudden death during coitus...	.....	Marble	..	Atheroma of artery	Atheroma of aorta

10. Ogle, 1867.....	26	M	Blacksmith	Rheumatic fever twelve years ago	Two and one half years ago, pain in back of the head followed in two weeks by numbness in the thumb and first two fingers of the right hand and pains in the back, arms and abdomen; similar attack a month ago with paralysis of the arm and dropped wrist; murmur at the apex; sudden death	Three 1. Left 2. Right 3. Right	..... Pea Slightly larger than a pea Cob-nut	.. 0 0 +	.....	Acute endocarditis of the aortic valve; aneurysmal pouches of the brachial artery; renal infarcts (?)
11. Buchner, 1867...	47	M	Painter	.....	For ten months anginal attacks at times associated with palpitation; more frequent in past six weeks; blowing murmur over the entire precordium; sudden death	Left	6 cm. in diameter	0	Dilatation of the proximal portion of left; bony plaques on the floor of the aneurysm	Dilatation of the right and posterior sinuses of Valsalva; fibrinopurulent pericarditis
12. Gee, 1871.....	7	M	.....	....	Died of scarlatinal dropsy with intercurrent pneumonia and meningitis	Three 1. Left 2. Right 3. Right	..... Pea Pea Horse-bean	.. 0 0 0	.....	Valves healthy; aorta near valves and aortic cusps of mitral presented specks of atheroma
13. Crisp, 1871.....	63	M	Carpenter	Prolonged submersion twenty years ago; fall from scaffold three years ago; intemperate	Large hematemesis three and one half months ago; sudden death	Right	Walnut	+	Moderate atheroma	Valves normal; slight atheroma of the aorta
14. Malet and Evans, 1887	5	M	.....	.....	For three weeks intense thirst and polyuria; pale and emaciated; two small unhealthy sores on the face and one on the leg; sudden death	Left	Pea	+	.....	Valves healthy and competent
15. Eppinger, 1887..	25	F	.....	Pain in the ankle joints and fever four years ago; since then symptoms of cardiac disease	For two months, sticking pains in the chest and abdomen; cough, fever, ascites and anasarca; systolic murmur at apex and over right side of the heart	Left	5 mm. long	0	Intima gone, elastica torn and inflammatory infiltration, especially of the adventitia	Chronic endocarditis of the aortic and mitral valves with fresh vegetations on the latter; aneurysm of the splenic artery; acute hemorrhagic nephritis

TABLE 2.—*Report of Cases of Aneurysm in the Literature—Continued*

Author	Age	Sex	Occupation	Previous History	Symptoms and Physical Conditions	Site	Size	Rupture	Coronaries	Additional Pathologic Changes
16. Clarke, 1896.....	10	F	.....	.....	One month ago, swelling half the size of a walnut on right buttock; three weeks later, similar lump in the left axilla; sudden death	Dozen on both	Pea to small chestnut	+	Inner coats of the coronaries and branches greatly thickened	Fibrous ring of thickening on the mitral, but no recent vegetations; aneurysm in left axilla not proved
17. Capps, 1889.....	About 40	M	.....	.....	Found dead in street.....	Left	Pigeon's egg	0	Anterior branch of left thickened; posterior branch calcified; slight atheromatosis of the right	Valves normal; slight atheromatosis of the aorta
18. Capps, 1899.....	39	M	Telegraph operator	Syphilis several years ago; one year ago, left hemiparesis; alcohol to excess	For four weeks, numbness and tingling in the legs and feet and later over the whole body; convulsions; died of bronchopneumonia	Two Left	1. Hazelnut 2. Smaller	0	Wall of the left coronary streaked with yellowish spots and calcareous scales	Syphilitic aortitis with early aneurysm; bulging of interventricular septum; aortic valves rough, thickened and sclerosed
19. Griffith, 1901....	19	F	.....	.....	Rheumatic pains in both knees one year ago; symptoms of nephritis nine months afterward; diagnosis of mitral disease; at that time symptoms of acute endocarditis	Right	Pea	0	Wall of the aneurysm tough and fibrous	Subacute bacterial endocarditis of the mitral and aortic valves
20. Griffith, 1901....	45	F	.....	Rheumatism at 14	Old mitral disease with heart failure	Right	Orange pip	0	Atheromatous patches in both and slight tortuosity	Subacute bacterial endocarditis of aortic and mitral valves; old infarcts of spleen and kidneys
21. Toller, 1904.....	40	M	Soldier	Rheumatic fever	Shortness of breath on exertion, cardiac pain with cough and occasional vomiting; a few weeks later slight ascites and transient edema of the eyelids; systolic murmur over the lower end of the sternum; developed generalized anasarca; paralysis of left vocal cord and consolidation of the right apex of the lung; gradual cardiac failure	Left	Hen's egg	0	Left negative below aneurysm; right sclerotic	Valves (?), concretio cordis

22. Ruge, 1905.....	12	M	.....	.....	Following abrasion of the left foot, lymphadenitis of the groin and osteomyelitis of the head of the left femur; in six weeks signs of cardiac disease and then of pyemia; hip joint resected	Left	Walnut	0	Intima of the aneurysm replaced by connective tissue; elastic fibers still visible; streptococci	Acute seropurulent pericarditis; valves normal; foramen ovale closed and veins of the lung normal
23. Winkler, 1908...	68	M	.....	.....	Had suffered from generalized arteriosclerosis and contracted kidneys	Left	.....	0	Both thickened and tortuous; much calcification	Scarring and brown atrophy of the heart muscle; marked arteriosclerosis of the aorta
24. Henke, 1910.....	..	..	.....	.....	.....	Left	Cherry	0	Septic embolus in the coronary; streptococci	Ulcerative endocarditis of the aortic valve; aneurysm of the mesenteric artery
25. Sommer, 1910...	73	M	Laborer	.....	.....	Left	Two peas	+	Slight atheromatosis	Valves normal; slight general arteriosclerosis, more marked in the cerebral arteries; hemorrhage into the right cerebral hemisphere
26. Martland, 1917	32	F	.....	.....	Vague epigastric and precordial pain; found dead	Right	6 cm. in diameter	+	.....	Syphilitic meso-aortitis with aneurysmal dilatation
27. Fraenkel, 1917..	20	M	Soldier	Wounded in the right arm and the head a year ago	Shortness of breath and anasarca	Left	Half size of cherry stone	0	Aneurysm completely filled with red thrombus	Aneurysmal projection of the left ventricular wall
28. Lublin, 1920.....	22	M	Soldier	Rheumatic fever at 9	Acute nephritis; systolic murmur at the apex; embolus to the right radial artery; sudden uremia	Left	Small	0	.....	Recurrent verrucous endocarditis of the aortic and mitral valves; left ventricular scar; splenic infarct
29. Windholz, 1926	62	F	.....	.....	Suffered for ten years with cardiac disease and died with symptoms of decompensation	Two Left	1. Nut 2. Pea	0	The first aneurysm, paper-thin walls with hyaline deposits; the second, rigid walls with calcified plaques	Chronic endocarditis of the aortic valve

Eppinger [14] believed that the mycotic embolus produced an acute inflammation of the intima at the point in which it came to rest and then spread to the adventitia by way of the vasa vasorum. From both these areas, the media next became involved, finally with a rupture of the internal elastic lamina. On the other hand, Benda [38] claimed that the process was an ulcerative endarteritis that invaded the media before reaching the adventitia. In all probability, both methods of progression can occur. Experimentally, the choice of route is apparently dependent on the virulence of the inflammation,[39] the subacute following the description of Eppinger's and the acute following those of Benda.

Arteriosclerotic Aneurysms.—We have classified the remaining cases under this heading for want of a better term and, in that, the only thing they have in common is an arteriosclerosis of the coronary arteries. In view of the extremely high incidence of coronary sclerosis and the rarity of aneurysm, this can hardly be conceived as being the only factor involved in their production. Again, it is the general consensus of opinion among pathologists that the etiologic importance of arteriosclerosis in the formation of aneurysms has been much overrated. A similar situation exists with regard to aneurysms of the cerebral arteries.

It seems to us, however, that the length of time in which a severe sclerosis has existed must not be overlooked in a consideration of this problem. That coronary sclerosis can commence at an early age—even in childhood—is generally conceded.[40] Mönckeberg [41] found arteriosclerotic foci in 40 per cent of soldiers between the ages of 18 and 20. It is also well known that sufferers from severe coronary sclerosis die within a relatively short time from myomalacia, angina pectoris or coronary thrombosis. Many investigators, however, such as Gross, Oberhelman and Le Count and Spalteholz, have shown that the coronary vessels are not end arteries and that anastomoses may exist between the various branches. Dependent on the richness of the preexistent anastamoses and the slowness of progression of the sclerosis, an advanced degree of the latter can be reached and still be compatible with well being. In fact, as occurred in one of our patients, a rupture of the heart itself took place without a previous history of morbidity. Given a severe sclerosis with marked degeneration and atrophy of the media plus a long period of increased intravascular tension it is interesting to speculate whether an aneurysm can develop slowly. The left

38. Benda: Ergebn. d. allg. Pathol. u. path. Anat. **8**:196, 1902.

39. Klotz: Brit. M. J. **1**:1767, 1906. McMeans: J. M. Research **32**:388, 1915. Richey, de W. G., and Maclachlan, W. W. G.: Mycotic Embolic Aneurysms of Peripheral Arteries, Arch. Int. Med. **29**:131 (Jan.) 1922.

40. Wiener: Wien. klin. Wchnschr. **19**:725, 1906. Wiesel: ibid. **19**:723, 1906.

41. Mönckeberg: Centralbl. f. Herzkrankh. u. Gefässkrankh. **8**:2, 1916.

coronary preponderance and the situation of the aneurysm immediately beyond the orifice are in agreement with this point of view.

Including our own, we have placed twelve cases in this group (Peste,[42] Peacock,[22] Wood,[43] Büchner,[44] Crisp,[3] Capps [4] [cases 1 and 2], Winkler,[45] Sommer,[27] Martland,[25] Windholz [28] and Packard). Of these, the condition involved the left coronary artery in nine, the right coronary artery in two, and in one, the site was not stated. In one case (Windholz), the left coronary artery was the seat of two aneurysms. Rupture had taken place in seven cases—five into the pericardial sac and two into the pulmonary artery. The average age was 57.

In the vast majority of cases, the coronary artery or arteries were described as being moderately or markedly tortuous and calcified. When the data are furnished, the wall of the aneurysm either is extremely thin and exhibits irregular hyaline deposits or is made rigid by scattered calcareous plaques.

In the few cases in which a histologic examination of the aneurysm was made, the usual picture of an advanced arteriosclerosis was found.

Pure Mycotic Aneurysms.—The pathologic processes in this type are practically identical with those of the mycotic-embolic aneurysms that have been described. Ruge reported a case of coronary aneurysm in a boy with a pyemia resulting from an osteomyelitis of the femur. The heart chambers, the valves and the pulmonary veins were normal, and there was no communication between the right and the left sections of the heart.

Traumatic Aneurysms.—Direct trauma is a rare cause of aneurysm in other arteries in the body. As is to be expected from their anatomic position, there is no example of it in our series. Whether indirect trauma can produce a tear in the intima of the coronary and a subsequent aneurysm is a moot question. Fraenkel considered his case as being of this nature. The aneurysm and the lumen of the coronary were filled with a red thrombus, and an aneurysmal bulging of the left ventricle was also present.

REPORT OF CASE

History.—I. S., a Jew, aged 60, a tailor, was admitted to the Gouverneur Hospital on Nov. 18, 1927. For the past two years, he had suffered with indigestion—occasional attacks of pain and distress in the epigastrium associated with nausea. On the morning of admission, he had had a typical anginal attack which, however, had passed off by the time the ambulance arrived. Examination revealed

42. Peste: Arch. gén. de méd. **2**:472, 1843.

43. Wood: New York M. J. **8**:384, 1860.

44. Büchner: Aneurysma der Arteria coronaria cordis sinistra, Amsterdam, C. G. Vander Post, 1867.

45. Winkler: Verhandl. d. deutsch. path. Gesellsch. **12**:195, 1908.

nothing but the presence of an inguinal hernia. He was brought to the hospital, and a few minutes later, the house physician found him dead in the admitting room.

Autopsy.—At autopsy, the pericardial sac was distended and contained about 350 cc. of fluid and clotted blood. The heart was enlarged, weighed 390 Gm. and measured 15 by 12 by 8 cm. The epicardial fat was somewhat increased in amount. Both ventricles were dilated, the right more so than the left. The left ventricular wall measured from 1.4 to 1.8 cm. in thickness and the right from 0.4 to 0.6 cm. The muscle was yellowish brown in color.

The mitral ring measured 12, the aortic 8.4, the tricuspid 14 and the pulmonary 8 cm. in circumference. Except for the dilatation of the mitral, tricuspid and pulmonary rings the valves were grossly normal.

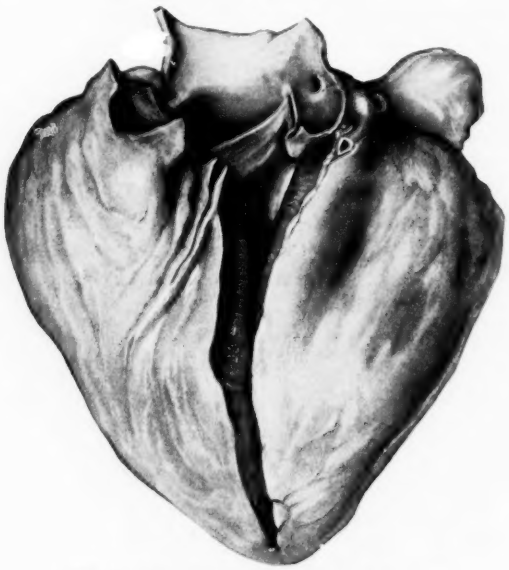

Fig. 1.—Aneurysm of left coronary artery.

The intima of the ascending aorta, near its origin, and especially about the commissures of the aortic valves, was cloudy and showed numerous white, hyaline, grayish and grayish blue, slightly raised areas. Many of these exhibited small depressions and wrinkles. The rest of the aorta showed many yellowish streaks and patches and a few calcified plaques.

In the periaortic space, between the auricles and surrounding the root of the aorta, there was a collection of dark, clotted blood.

The coronary orifices were normal in size. Both coronaries, especially the left, were markedly sclerotic and tortuous. Situated on the left coronary artery, 1.3 cm. from the orifice, there was a small globular protuberance about the size of a pea (fig. 1). It lay in the auriculoventricular groove between the aorta and the left auricular appendix. On its surface, there was a small teatlike process covered by a blood clot.

When an opening was made into the vessels, the lumens were found to be patent. The atheroma and calcification commenced immediately beyond the orifices and extended down into their main branches. The protuberance on the left coronary was found to be a small aneurysmal pouch, the floor of which exhibited changes similar to those found in the rest of the artery. A probe, which was inserted into the sac, passed into the teatlike process and through a tiny perforation out into the periaortic space.

Fig. 2.—Syphilitic mesoaortitis; × 250.

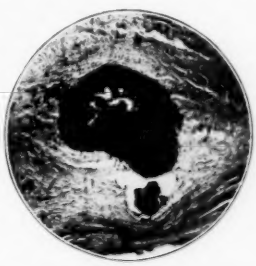

Fig. 3.—Coronary sclerosis; × 250.

Besides hyaline and fatty degeneration of the intima, the aorta exhibited a marked round cell infiltration of the adventitia, most marked about the vasa vasorum. With the latter, it had invaded the media and produced irregularly strewn strands of scar tissue rich in wandering cells (fig. 2).

The coronary vessels showed a marked atherosclerosis, with a pronounced thickening of the intima by dense, homogeneous, hyaline, connective tissue, which overlapped the area of fatty degeneration. Extensive calcification of these degenerated and necrotic foci had taken place. The internal elastic lamella was thin and frayed out at the edges of the plaques. The media was thin and atrophic (fig. 3).

The heart muscle showed moderate degenerative changes and a slight interstitial fibrosis. The lungs were normal except for emphysema. The gastro-intestinal tract showed nothing of interest. The liver was somewhat enlarged, weighed 1,800 Gm. and was fatty. The spleen was adherent to the surrounding structures; the capsule was thickened, weighed 150 Gm. and was firmer than normal. Microscopically, the fibrous septums were thickened, and the reticular tissue was increased in amount.

The kidneys were slightly reduced in size, each weighing 125 Gm. The capsule stripped with some difficulty, leaving a slightly granular surface. On section, the cortex was somewhat diminished in size, and the markings were indistinct. The blood vessels were prominent. Microscopically, the cortex contained scattered patches of fibrosis, in which the glomeruli were changed into dense hyaline nodules. The larger vessels were sclerotic, and many of the arterioles showed hyaline deposits beneath the endothelium. A moderate round cell infiltration was present in the fibrotic areas. The pancreas, suprarenals and testicles were grossly normal. The skull was not opened.

Anatomic Diagnosis.—A diagnosis was: hemopericardium, ruptured aneurysm of the left coronary artery, coronary sclerosis, atherosclerosis and syphilitic meso-aortitis of the aorta, hypertrophy and dilatation of the left ventricle, dilatation of the right ventricle, pulmonary emphysema, fatty degeneration of the liver, chronic splenitis and renal arteriosclerosis.

SUMMARY

1. Aneurysm of the coronary arteries is an extreme rarity. This study is based on the records of twenty-nine cases gathered from the literature and one here reported.

2. The condition is three times as common in males as in females.

3. In the vast majority of cases, the aneurysm is single and is usually situated on the left coronary artery within the first inch of its course.

4. Rupture of the aneurysm took place in 50 per cent of the cases.

5. Etiologically, the cases can be divided into two main groups: mycotic-embolic and arteriosclerotic.

6. The mycotic-embolic aneurysms occurred in association with an acute or subacute bacterial endocarditis of the aortic valve. The average age was 27.

7. In the arteriosclerotic group, a marked coronary sclerosis was present; the average age was 57.

8. Syphilitic meso-aortitis was found in only three cases in this series.

9. Trauma plays an insignificant etiologic rôle.

10. There were not any pathognomonic symptoms or physical signs which made possible an antemortem diagnosis of the condition.

THE EFFECT OF ROENTGEN RAYS ON THE HEART

II. THE MICROSCOPIC CHANGES IN THE HEART MUSCLE OF RATS AND OF RABBITS FOLLOWING A SERIES OF EXPOSURES *

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In a previous article,[1] we came to the conclusion that the single exposure of the precordium to roentgen rays of the quality and quantity described in that paper, and corresponding to a so-called human erythema dose, does not produce definite and irreparable injury to the heart muscles of adult rats and rabbits. The next step in these investigations suggested itself, i. e., the study of the effect of a series of exposures as they are carried out in the treatment of human beings. All malignant lesions in the region of the heart, for instance, mediastinal growth, carcinoma of the left breast, bronchus or a tumor of the lung are repeatedly treated at certain intervals with a single dose, varying from a part to a full tolerance dose. The latter term is used in preference to the "erythema dose," which implies a unit, but careful investigations have shown that it is not reproducible.[2]

TECHNIC

The technical procedure was the same as in the previously reported experiments. Two types of radiation were used. In the first type the dosage was: 200 kilovolts, 25 milliamperes, 40 cm. focal skin distance, 0.5 copper plus 1 aluminum filter; time of exposure, nine minutes; lambda effective equaled 0.16 angstrom units in copper. The human skin tolerance dose was taken at 600 R as measured with the ionization chamber in air, representing, therefore, primary energy.[3] This value is called the roentgen value. In the second type of radiation, the dosage was: 130 kilovolts, 5 milliamperes, 24 cm. focal skin distance, 4 aluminum filter;

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* Read before the Section on Radiology at the Seventy-Ninth Annual Session of the American Medical Association, Minneapolis, June 14, 1928.

1. Warthin, A. S., and Pohle, E. A.: The Effect of Roentgen Rays on the Heart: I. The Microscopic Changes in the Heart Muscle of Rats and Rabbits Following a Single Exposure, J. A. M. A. **89**:1825 (Nov. 26) 1927.

2. Pohle, E. A., and Barnes, J. M.: Clinical and Physical Investigations of the Problem of Dosimetry in Roentgen Therapy, Radiology **10**:303, 1928.

3. As small fields were used, it may be assumed that the surface dose is identical with this value.

time of exposure, six minutes; lambda effective equaled 0.23 angstrom units in copper. The roentgen value was approximately 300 R. The figures given in the first paper for the roentgen value, namely, 1,100 R, should be corrected to about 660 R, because the discrepancy which existed at that time between the so-called German and the American unit has been removed; the two units are now identical.

In all our measurements of the output of the apparatus and tube, a Wulf ionometer calibrated in roentgen units served as an ionization instrument. Its sensitivity was checked by radium. The absolute standard for the roentgen unit was obtained by means of an open air ionization chamber in connection with a high sensitive galvanometer as suggested by Duane and Lorenz.[4]

Twenty-four rabbits and forty-eight rats, leaving two or three controls in each group of twelve animals, received treatment once a month for three months.[5] The exposures were arranged so that an initial dose of 600 R over the precordium was followed by 2×300 R in monthly intervals. In order to guarantee the correct position of the heart during the exposure, diagnostic films were taken in every case before treatment was begun (fig. 1). The animals were killed from eight to thirty-two days after the last exposure, and the hearts were taken out and prepared for microscopic examination.

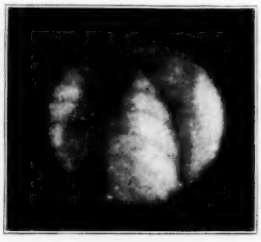

Fig. 1.—Roentgenogram of the heart taken before treatment.

PROTOCOLS OF EXPERIMENTS

Rat 1 (series I, 4387-L-AF).—Killed 1-21-28. Well-marked Zenker's necrosis. Moderate fatty degenerative infiltration most marked beneath the epicardium. Endothelium of some of the smaller blood vessels showed swelling and vacuolation. The ganglion cells of the intracardiac nodes showed a well-marked chromatolysis. (Severe purulent bronchitis, particularly of the larger bronchi. Areas of purulent bronchopneumonia.)

Rat 2 (series I, 4379-L-AF).—Killed 1-21-28. Well-marked cloudy swelling and Zenker's necrosis. Fatty degenerative infiltration more marked than in rat 1, and particularly beneath the endocardium, but also well-marked beneath the epicardium. Changes more marked than in rat 1. Marked congestion of the small vessels and capillaries, particularly in the areas showing the most marked Zenker's necrosis. Pyknosis of many of the endothelial nuclei. Many nuclei of the valvular endocardium showed pyknosis. The ganglion cells of the intracardiac nodes showed a well-marked chromatolysis. (Severe purulent bronchitis, particularly of larger bronchi. Areas of purulent bronchopneumonia.)

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4. Duane, W., and Lorenz, E.: The Standard Ionization Chamber for Roentgen-Ray Dosage Measurements, Am. J. Roentgenol. **19**:461, 1928.

5. Mr. W. Thayer assisted in carrying out the animal experiments.

RAT 3 (series I, 4380-L-AF).—Killed 1-21-28. Changes similar in kind but to a much less marked degree as compared to rats 1 and 2. (Severe purulent bronchitis, particularly of the larger bronchi. Areas of purulent bronchopneumonia.)

RAT 4 (series I, 4381-L-AF).—Killed 1-21-28. Marked Zenker's necrosis. Moderate fatty degenerative infiltration beneath epicardium and endocardium. Numerous pyknotic endothelial nuclei. No ganglion cells in sections examined. (Severe purulent bronchitis, particularly of the larger bronchi. Areas of purulent bronchopneumonia.)

RAT 5 (series I, 4382-L-AF.)—Killed 1-21-28. Marked Zenker's necrosis with fatty degenerative infiltration most marked on the endocardial side. Numerous pyknotic endothelial nuclei. In the adipose tissue at the base of the heart there were small areas of necrosis with inflammatory reaction. Early localized epicarditis. Ganglion cells showed marked chromatolysis. (Severe purulent bronchitis, particularly of the larger bronchi. Areas of purulent bronchopneumonia.)

RAT 6 (series I, 4383-L-AF).—Killed 1-21-28. Well-marked Zenker's necrosis. Fatty degenerative infiltration beneath epicardium and endocardium. Scattered pyknotic nuclei. Early epicarditis, localized toward the base. Areas of inflammatory infiltration through the adipose tissue. Degenerative changes in the ganglion cells. (Severe purulent bronchitis, particularly of the larger bronchi. Areas of purulent bronchopneumonia.)

RAT 7 (series I, 4384-L-AF).—Killed 1-21-28. Moderate Zenker's necrosis. Fatty degenerative infiltration. Scattered pyknosis. No ganglion cells in sections examined. Ganglion cells showed the same degree of chromatolysis. No epicarditis. (Severe purulent bronchitis, particularly of the larger bronchi. Purulent bronchopneumonia.)

RAT 8 (series I, 385-L-AF).—Killed 1-21-28. Marked Zenker's necrosis, more so than in preceding protocol. Numerous pyknotic endothelial and heart muscle nuclei. Subepicardial and subendocardial fatty degenerative infiltration. Slight increases of lymphocytes around some of the blood vessels. Areas of marked Zenker's necrosis in the myocardium of the auricle. Localized epicarditis. Some degree of chromatolysis of ganglion cells as in the preceding protocol. (Severe purulent bronchitis, particularly of the larger bronchi. Areas of purulent bronchopneumonia.)

RAT 9 (series I, 4386-L-AF).—Killed 1-21-28. Marked Zenker's necrosis particularly of the inner half of the left ventricle. Fatty degenerative infiltration, both subendocardial and subepicardial. Scattered pyknotic endothelial and myocardial nuclei. Ganglion cells showed the same degree of chromatolysis. Pyknotic nuclei observed in one nerve trunk. Numerous large "mast" cells in the adipose tissue about the ganglions. (Severe purulent bronchitis, particularly of the larger bronchi. Areas of purulent bronchopneumonia.)

RAT 10 (series I, 4387-L-AF).—Control; killed 1-21-28. Early Zenker's necrosis and fatty degenerative infiltration, less marked than in preceding protocol. Milder degree of bronchitis and bronchopneumonia.

RAT 11 (series I, 4388-L-AF).—Control; killed 1-21-28. Changes similar to the preceding protocol, but less severe. Early purulent bronchopneumonia.

RAT 12 (series I, 4389-L-AF).—Control; killed 1-21-28. Well-marked Zenker's and fatty degenerative infiltration. Numerous pyknotic nuclei. Increase of interstitial nuclei in many areas. Areas of fat necrosis at base of heart with beginning inflammatory reaction. Early purulent mediastinitis.

Rat 1 (series II, 4449-L-AF).—Killed 1-28-28. Well-marked Zenker's necrosis beneath the epicardium, more marked beneath the endocardium. Numerous pyknotic nuclei. Marked chromatolysis of ganglion cells. (Purulent bronchitis of large bronchus.)

Rat 2 (series II, 4450-L-AF).—Killed 1-28-28. Marked Zenker's necrosis. Subepicardial and subendocardial fatty degenerative infiltration. Many ganglion

Fig. 2 (series II, rat 4).—Marked fatty degenerative infiltration and Zenker's necrosis of the muscle of the auricle and the right ventricle; marked Zenker's necrosis of the left ventricle.

cells showed karyolysis and others showed marked chromatolysis. (No lung tissue examined.)

Rat 3 (series II, 4451-L-AF).—Killed 1-28-28. Marked Zenker's necrosis. Subepicardial and subendocardial fatty degenerative infiltration. (Severe purulent bronchopneumonia.) Edema of nerve trunks. Chromatolysis of ganglion cells.

Rat 4 (series II, 4452-L-AF).—Killed 1-28-28. Marked fatty degenerative infiltration and Zenker's necrosis of muscle of auricle and right ventricle. Marked

Zenker's necrosis of left ventricle. Numerous pyknotic nuclei. (Acute catarrhal bronchitis.) Edema of large nerve trunk. No ganglion cells included in sections examined.

RAT 5 (series II, 4453-L-AF).—Killed 1-28-28. Ganglions showed marked chromatolysis. Myocardium showed marked Zenker's necrosis and fatty degenerative infiltration most marked in auricle and right ventricle. (Acute catarrhal bronchitis.)

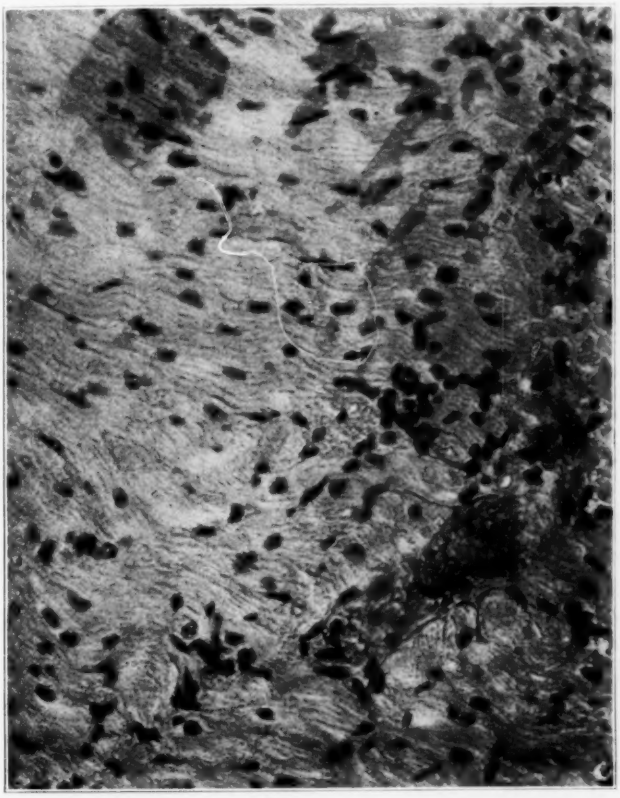

Fig. 3 (series IV, rat 5).—Marked Zenker's necrosis; small areas of pyknotic nuclei.

RAT 6 (series II, 4454-L-AF).—Killed 1-28-28. Well-marked Zenker's necrosis. Fatty degenerative infiltration. Edema of nerve trunks. Chromatolysis of ganglion cells. (Very severe purulent bronchitis and bronchopneumonia.) Early epicarditis, Small areas of interstitial myocarditis.

RAT 7 (series II, 4455-L-AF).—Killed 1-28-28. Marked Zenker's necrosis. Subepicardial and subendocardial fatty degenerative infiltration. Pyknosis of nuclei. Increase of interstitial cells in heart muscle. Marked edema and degeneration of large nerve trunks. Chromatolysis of ganglion cells.

Rat 8 (series II, 4456-L-AF).—Killed 1-28-28. Marked Zenker's necrosis. Subendocardial fatty degenerative infiltration. Marked fatty degenerative infiltration of right auricle and right ventricle. Marked chromatolysis of ganglion cells. Complete destruction of nuclei. (Purulent bronchopneumonia.) Lymph nodes largely replaced by cells resembling myelocytes or plasma cells. In the sinuses of the nodes large basophilic reticulo-endothelial cells.

Rat 9 (series II, 4457-L-AF).—Killed 1-28-28. Marked Zenker's necrosis. Subepicardial and subendocardial fatty degenerative infiltration, more marked in

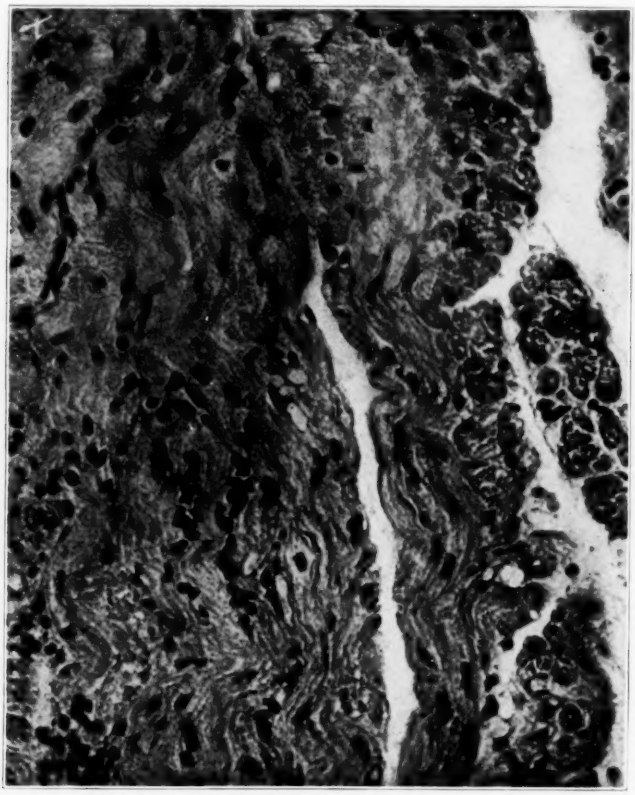

Fig. 4 (series I, rat 4).—Marked Zenker's necrosis; moderate fatty degenerative infiltration beneath epicardium and endocardium.

right ventricle than in the left. Acute epicarditis. Chromatolysis of ganglion cells. (Purulent bronchopneumonia.)

Rat 10 (series II, 4458-L-AF).—Died 1-12-28. Marked Zenker's necrosis. Fatty degenerative infiltration both subepicardial and subendocardial. Localized increase of interstitial nuclei. (No lung tissue included.) No ganglions found in sections examined.

RAT 11 (series II, 4459-L-AF).—Control; killed 1-28-28. (Purulent bronchopneumonia.) Marked Zenker's necrosis. Subepicardial and subendocardial fatty degenerative infiltration. Edema of nerve trunks. Early pleuritis. Chromatolysis of ganglion cells.

RABBIT 1 (series I, 6093-L-AF).—Died 3-4-28. Marked Zenker's and simple necrosis without inflammatory reaction. Large red thrombus in left ventricle. Suffusion with hemoglobin (post mortem?). No pneumonia. Catarrhal bronchitis

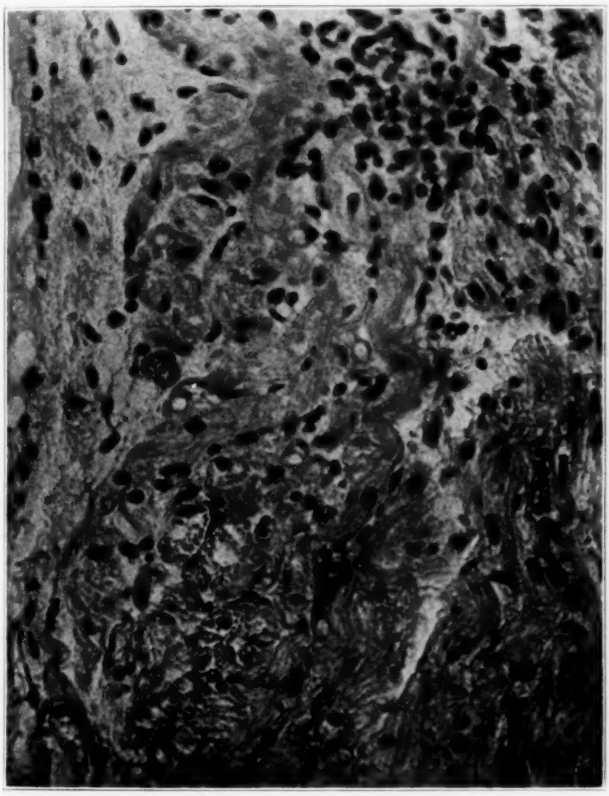

Fig. 5 (series I, rat 5).—Marked Zenker's necrosis with fatty degenerative infiltration most marked on the endocardial side.

of the larger bronchi. Hyperplasia of the reticulo-endothelium of the bronchial lymph nodes.

RABBIT 3 (series I, 6094-L-AF).—Killed 3-22-28. Moderate Zenker's necrosis. Marked fatty degenerative infiltration beneath endocardium, particularly of the papillary muscles. Pyknosis of interstitial nuclei. Some localized increase of interstitial nuclei. Small areas of definite interstitial myocarditis in muscle near base of heart. Fatty change is more marked in the auricular wall than elsewhere. Catarrhal bronchitis.

Rabbit 4 (series I, 6095-L-AF).—Killed 3-22-28. Zenker's necrosis and marked fatty degenerative infiltration. Localized increase of the interstitial nuclei. Marked lipoidosis of endocardium of aortic valve. There were areas scattered throughout the wall of the heart of definite interstitial inflammation and repair of focal necrosis. Endocardium near these areas showed a marked lipoidosis. Nerve trunks showed no change. Early epicarditis. No ganglions found. Catarrhal bronchitis.

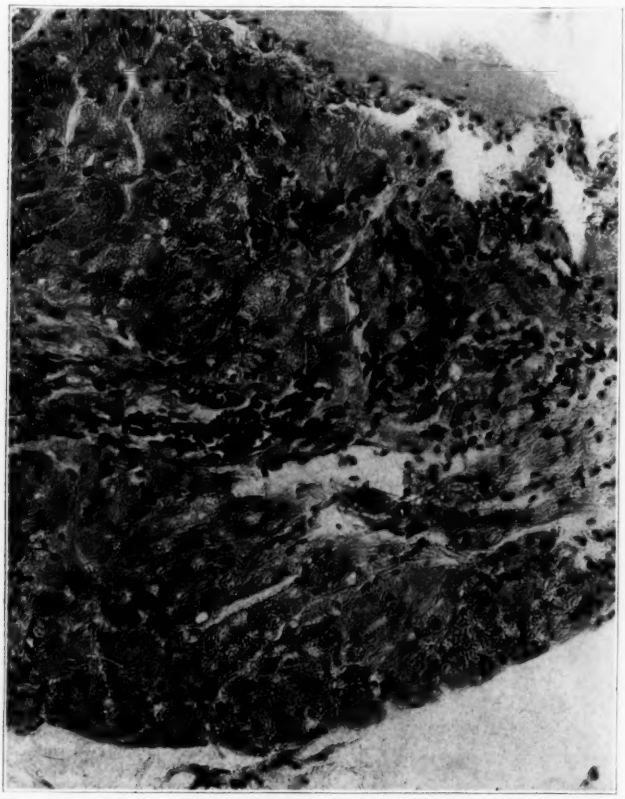

Fig. 6 (series I, rat 6).—Well-marked Zenker's necrosis; fatty degenerative infiltration beneath epicardium and endocardium.

Rabbit 5 (series I, 6096-L-AF).—Killed 3-22-28. Zenker's necrosis. Marked subendocardial fatty degenerative infiltration. Numerous pyknotic interstitial nuclei. Small areas of small-celled infiltration, more marked in the auricular walls than in the ventricle. Focal areas of complete necrosis of heart muscle with beginning repair. No ganglions found. Catarrhal bronchitis in main trunks.

Rabbit 6 (series I, 6097-L-AF).—Killed 3-22-28. Zenker's necrosis. Diffuse fatty degenerative infiltration, more marked beneath the endocardium and in the

papillary muscles. Numerous small areas of increased interstitial nuclei, lymphocyte infiltration. Some of these areas showed pyknotic nuclei. Nerve trunks negative. Definite inflammatory infiltrations in auricular wall. Auricular ganglion showed marked chromatolysis. Catarrhal bronchitis.

RABBIT 7 (series I, 6098-L-AF).—Killed 3-22-28. Marked Zenker's necrosis. Fatty degenerative infiltration most marked beneath the endocardium. Small areas

Fig. 7 (series III, rat 2).—Extreme Zenker's necrosis.

of lymphocyte infiltration and increase of interstitial nuclei. No ganglion found. Nerve trunks negative. Catarrhal bronchitis in large bronchi.

RABBIT 8 (series I, 6099-L-AF).—Died 2-24-28. Marked Zenker's necrosis and fatty degenerative infiltration, but few normal muscle fibers. Very slight increase of interstitial nuclei. Auricular wall showed marked fatty degenerative infiltration. Catarrhal bronchitis in large bronchi.

RABBIT 9 (series I, 6100-L-AF).—Killed 3-22-28. Marked Zenker's necrosis and fatty degenerative infiltration. Very small aggregations of lymphocytes and

interstitial nuclei. Nerve trunks showed no changes except that one has a lymphocytic infiltration along its intraneural blood vessel. No ganglion found. Catarrhal bronchitis.

RABBIT 10 (series I, 6101-L-AF).—Killed 3-22-28. Marked Zenker's necrosis and fatty degenerative infiltration. Localized increase of interstitial nuclei. Numerous small lymphocyte infiltrations. Changes most marked in auricle and wall of ventricle. Large areas of marked Zenker's necrosis in wall of right

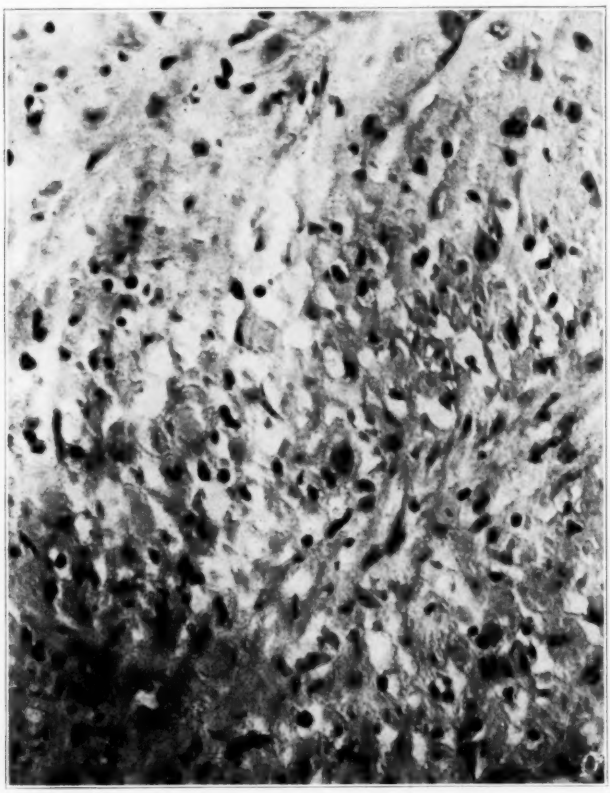

Fig. 8 (series I, rabbit 4).—Zenker's necrosis and marked fatty degenerative infiltration.

auricle and ventricle. Early inflammatory reaction and some repair. No ganglions found. Purulent catarrh of large bronchi.

RABBIT 11 (series I, 6091-L-AF).—Control; killed 3-22-28. No Zenker's necrosis. Heart showed fatty infiltration and a diffuse fatty degenerative infiltration. Early atherosclerosis of aorta. No pneumonia.

RABBIT 12 (series I, 6092-L-AF).—Control; killed 3-22-28. No Zenker's necrosis. Diffuse fatty degenerative infiltration, more marked beneath the endocar-

dium. Subepicardial fatty infiltration. Slight lipoidosis of intima of aorta. (Nodules of cartilage in the aortic valve. Old atherosclerosis of this valve.) No pneumonia.

RAT 1 (series III, 7004-L-AF).—Died 3-5-28. Extreme Zenker's necrosis of heart muscle. (Multiple abscesses in lung. Caseation of pus. Purulent bronchopneumonia.)

RAT 2 (series III, 7007-L-AF).—Killed 5-2-28. Extreme Zenker's necrosis. No reactions.

RAT 3 (series III, 7005-L-AF).—Died 4-18-28. Extreme Zenker's necrosis. Very little fatty degenerative infiltration. No reactions. Necrosis of ganglion cells. Purulent bronchopneumonia.

RAT 4 (series III, 7009-L-AF).—Died 4-30-28. Marked Zenker's necrosis. Parietal mixed thrombus with karyorrhexis of the leukocyte nuclei. Marked chromatolysis of the ganglion cells. In the cardiac thrombus many colonies of bacteria. Thrombo-endocarditis purulenta. The thrombus rests on a completely necrotic endocardium and myocardium.

RAT 5 (series III, 7008-L-AF).—Killed 5-2-28. Marked Zenker's necrosis, slightly less marked than in the preceding protocol. No reactions. Catarrhal bronchitis.

RAT 6 (series III, 7006-L-AF).—Died 4-25-28. Extreme Zenker's necrosis. Marked necrosis of ganglion cells. No reactions. Purulent bronchopneumonia.

RAT 7 (series III, 7010-L-AF).—Killed 5-2-28. Moderate Zenker's necrosis. No reaction. Catarrhal bronchitis.

RAT 8 (series III, 7011-L-AF).—Killed 5-2-28. Marked Zenker's necrosis. No reactions.

RAT 9 (series III, 7012-L-AF).—Killed 5-2-28. Marked Zenker's necrosis. No reactions. (Diffuse purulent pneumonia.)

RAT 10 (series III, 7013-L-AF).—Control; killed 5-2-28. Marked Zenker's necrosis. Small localized increase of stroma cells. No ganglion found. Purulent bronchopneumonia.

RAT 11 (series III, 7014-L-AF).—Control; killed 5-2-28. Same as preceding protocol.

RAT 12 (series III, 7015-L-AF).—Control; killed 5-2-28. Marked Zenker's necrosis. Small localized areas of inflammation. Chromatolysis of ganglion cells. Purulent bronchopneumonia.

RAT 1 (series IV, 7016-L-AF).—Killed 5-2-28. Marked Zenker's necrosis. No reaction. No pneumonia.

RAT 2 (series IV, 7017-L-AF).—Killed 5-2-28. Marked Zenker's necrosis. Chromatolysis of ganglion cells. No reactions. No pneumonia.

RAT 3 (series IV, 7018-L-AF).—Killed 5-2-28. Marked Zenker's necrosis. Marked edema of nerve trunks. Marked chromatolysis of ganglion cells. No reactions. No pneumonia.

RAT 4 (series IV, 7019-L-AF).—Killed 5-2-28. Marked Zenker's necrosis. No reactions. Catarrhal pneumonia.

RAT 5 (series IV, 7020-L-AF).—Killed 5-2-28. Marked Zenker's necrosis. Small areas of pyknotic nuclei. (Small areas of pneumonia.) No reactions. Ganglion cells not found.

RAT 6 (series IV, 7021-L-AF).—Killed 5-2-28. Marked Zenker's necrosis. No reactions. (Acute catarrhal bronchitis.)

Rat 7 (series IV, 7022-L-AF).—Killed 5-2-28. Marked Zenker's necrosis. Small areas of pyknotic nuclei and lymphocyte infiltration. No pneumonia.

Rat 8 (series IV, 7023-L-AF).—Killed 4-30-28. (Severe necrotic hemorrhagic pneumonia.) Marked Zenker's necrosis of myocardium. (Marked purulent bronchitis of larger bronchi. Purulent mediastinitis.) Very marked necrosis of auricular wall.

Fig. 9 (series I, rabbit 6).—Zenker's necrosis; diffuse fatty degenerative infiltration, more marked beneath the endocardium and in the papillary muscles.

Rat 9 (series IV, 7024-L-AF).—Killed 5-2-28. Marked Zenker's necrosis. A few small reactions. No ganglions found. Catarrhal bronchitis.

Rat 10 (series IV, 7025-L-AF).—Control; killed 5-2-28. Zenker's necrosis less marked than in preceding protocol. No reactions. Catarrhal bronchitis.

Rat 11 (series IV, 7026-L-AF).—Control; killed 5-2-28. Marked Zenker's necrosis. No reactions. Purulent bronchopneumonia.

Rat 12 (series IV, 7027-L-AF).—Control; killed 5-2-28. Well-marked Zenker's necrosis. No reactions. Bronchopneumonia.

RABBIT 1 (series II, 7137-L-AF).—Killed 5-15-28. Moderate Zenker's necrosis with diffuse fatty degenerative infiltration. Nerve trunks negative. Ganglions not found. No reactions. No pneumonia.

RABBIT 2 (series II, 7138-L-AF).—Killed 5-15-28. Diffuse fatty degenerative infiltrations. Very slight Zenker's necrosis. Endocardial thickenings with myxomatous change. No pneumonia.

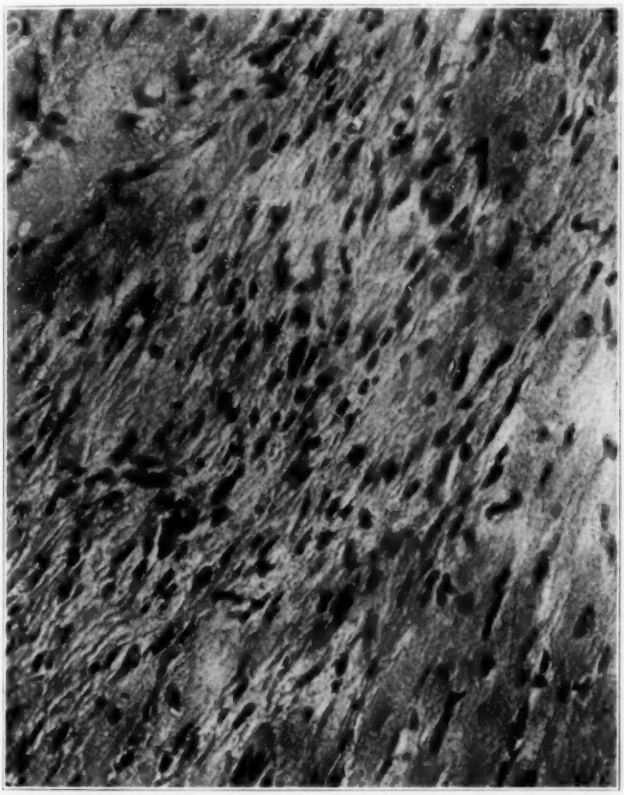

Fig. 10 (series I, rabbit 10).—Marked Zenker's necrosis and fatty degenerative infiltration.

RABBIT 3 (series II, 7139-L-AF).—Died 3-27-28. Marked atrophy of heart muscle. Relative increase of stroma and nuclei. Myxomatous thickening of endocardium. Marked serous atrophy of subepicardial fat. Fatty degenerative infiltration but no Zenker's necrosis. No pneumonia.

RABBIT 4 (series II, 7140-L-AF).—Killed 5-15-28. Atrophy of heart muscle. Well-marked Zenker's necrosis. Fatty degenerative infiltration most marked beneath the endocardium. Large areas of well-marked myocarditis. Increase of interstitial nuclei throughout. Catarrhal bronchitis in large bronchi.

Rabbit 5 (series II, 7141-L-AF).—Killed 5-15-28. Atrophy with relative increase of stroma nuclei. Subepicardial and subendocardial fatty degenerative infiltration. Small inflammatory reactions. Myxomatous thickening of endocardium. Catarrhal bronchitis in large bronchi.

Rabbit 6 (series II, 7142-L-AF).—Killed 5-15-28. Atrophy. Zenker's necrosis. Fatty degenerative infiltration, most marked beneath the endocardium.

Fig. 11 (series III, rat 6).—Extreme Zenker's necrosis; marked necrosis of ganglion cells.

Increase of stroma nuclei. Small areas of inflammatory infiltrations. Acute purulent epicarditis. Marked atrophy of thymus. Myxomatous thickening of endocardium. Localized perivascular infiltrations. Purulent bronchitis.

Rabbit 8 (series II, 7143-L-AF).—Killed 5-15-28. Zenker's necrosis. Diffuse fatty degenerative infiltration most marked beneath the endocardium. Localized increase of nuclei and small lymphocyte infiltrations. Marked thymic atrophy. Catarrhal bronchitis in large bronchi.

RABBIT 10 (series II, 7144-L-AF).—Control; killed 5-15-28. Atrophy. Zenker's necrosis. Fatty degenerative infiltration, most marked beneath the endocardium. Increase of stroma. Localized inflammatory infiltrations. Characteristic of "rabbit encephalitis."

RABBIT 11 (series II, 7145-L-AF).—Control; killed 5-15-28. Abnormal. Atrophy. Zenker's necrosis. Subendocardial fatty degenerative infiltration, marked in the papillary muscles. Relative increase of stroma cells. Small lymphocyte infiltrations. (Rabbit encephalitis?)

RABBIT 12 (series II, 7146-L-AF).—Control; killed 5-15-28. Marked Zenker's necrosis. Fatty degenerative infiltration, most marked beneath the endocardium. Relative increase of stroma. Small lymphocyte infiltrations. Nerve trunks are negative. No ganglion cells found. Acute epicarditis. (Purulent bronchopneumonia.) Coronary vessels showed some sclerosis. (Encephalitis?)

COMMENT ON PATHOLOGIC CHANGES CAUSED BY THE ROENTGEN RAYS

All of the irradiated animals (rats and rabbits) of these series showed marked myocardial lesions. The lesions consisted essentially in more severe degrees of Zenker's hyaline degeneration and necrosis and of fatty degenerative infiltration (lipoidosis) than were observed in the animals described in our first paper as following a single exposure. Furthermore, many of the animals showed varying degrees of inflammatory reactions and beginning interstitial proliferation. In general, the pathologic changes in the myocardium observed in these animals can be compared with lesions seen in the human heart in moderately severe and in severe diphtheria toxemia.

The exact interpretation of these lesions, so far as their production by irradiation is concerned, is rendered difficult by the complicating occurrence of a more or less severe purulent or catarrhal bronchitis and bronchopneumonia. The main bronchial trunks and the portions of the lungs in the anterior mediastinum were especially involved. The severity of these pulmonary lesions varied from a catarrhal bronchopneumonia to the occurrence of multiple pulmonary abscesses. There were also associated varying degrees of local or generalized mediastinitis, early pericarditis and pleuritis. In two of the animals (rat 4, series III and rabbit 1, series I), there was also a thrombo-endocarditis present. Bacterial colonies were seen in all of the lesions, so that there is no doubt as to the ultimate infective nature of these processes. Unfortunately, similar pulmonary lesions were found in the majority of the controls, and in some of these the same type of myocardial lesions of a less severe degree, with the exception of two cases among the control rats (series II, rat 11, series IV, rat 11) and in three control rabbits (series II, rabbits 10, 11 and 12). In addition to the pulmonary infection, these three animals showed myocardial lesions common to "rabbit encephalitis." With this complication, it becomes difficult to evaluate the actual rôle of irradiation in contributing to the myocardial damage. As some of the animals (control rats, series IV, rats 10 and 12, series I,

rats 10 and 11, and control rabbits, series I, rabbits 11 and 12) showed much milder myocardial lesions in spite of the associated pulmonary involvement, and because the controls with bronchopneumonia and myocardial lesions usually presented milder degrees of the latter, it seems safe to conclude that the heart lesions, in large measure at least, were due to the irradiation. This deduction is more safely drawn concerning the rats than concerning the rabbits because of the complicating heart lesions of rabbit encephalitis found in the rabbit controls. For this reason, we regard the rabbit as highly unsuitable for this especial research; it seems that the great majority of laboratory rabbits obtained at the University of Michigan show spontaneous myocardial lesions. The focal lymphocytic character of these lesions, however, can be used as a differential point in evaluating myocardial change due to other factors.

Carefully weighing the evidence, we feel that we are justified in concluding that the exposures given to these animals produced a primary mediastinal injury involving the heart, large bronchi, portions of the lungs, lymph nodes, thymus and ganglions. As a result of this primary injury, an infective bronchitis and bronchopneumonia followed in every exposed subject, frequently extending to pleurae, mediastinum and pericardium and in two cases associated with hematogenous bacterial thrombo-endocarditis. The lymph nodes and thymus of the region showed evidence of lymphoid destruction and reticulo-endothelial hyperplasia; areas of fat necrosis were present in the mediastinal fat, and the ganglions of the region showed marked chromatolysis and lipoidosis. Many nerve trunks were markedly edematous, and some showed lipoidosis. Some of these lesions did not have any relationship to the pneumonia, and were not due to infection. They occurred only in the irradiated animals. The changes common to infection were as a rule much more severe in the irradiated animals than in the controls dying with the severest form of pulmonary infection, even when in the rabbit controls associated with the lesions of rabbit encephalitis.

Although our animals were most carefully selected, and were kept under the very best conditions, a bronchial and pulmonary infection was practically universal among them. It seems probable that this began in the irradiated animals. As the result of the irradiation, the bronchi are damaged, then resistance lowered, and an infective bronchitis and bronchopneumonia developed. This in turn will increase the damage already produced by the irradiation in the myocardium, and the combination is sufficient to cause the spontaneous death of some animals. Since the controls were kept with the irradiated animals, the conditions favored the spread of the infection to them from the latter. This fact is of great importance; the main bronchial trunks are injured in mediastinal irradiation (repeated exposures) to such a degree that secondary pulmonary infection almost invariably results. To the pri-

mary myocardial injury is added the possibility of increased toxic injury and mechanical strain on the heart. The secondary infection may extend directly to the pericardium and heart, or the latter may be involved by hematogenous infection of the endocardium (thromboendocarditis). The injury to the nervous apparatus (ganglions) must cause added embarrassment to the circulation.

Comparing these observations with those of Hartman, Bolliger, Doub, and Smith,[6] we confirm their conclusion as to the possibility of injury to the myocardium by roentgen rays of short wave length. Unfortunately, no direct comparison of these two investigations is possible, since the foregoing authors did not make exact measurements of the applied dose. From an approximate estimate of the energy used by them, it would appear that the smallest exposure given by them must have been a multiple of our tolerance dose. There is no doubt that a dose of that order of magnitude must have a destructive effect on living tissue. As far as the human cases reported by them are concerned, no dose can be computed from the data given in the report, and the pathologic observations are not conclusively differentiated. There is no positive proof that the myocardial lesions were due to the irradiation. As far as our own clinical experience is concerned, no roentgen-ray injury of the heart was observed at autopsy in patients who had been treated over the precordium.

That an overdose of radium can lead to necrosis of the myocardium has been shown recently by E. Renfer.[7] In two cases of esophageal carcinoma in which treatment by radium was used (1968 and 2160 mgh.), he observed deep necrosis of the myocardium, in one case leading to rupture of the left ventricle and hemopericardium, with a latent period of from five to six months.

As Levy and Golden [8] have attempted to treat persons with heart disease (rheumatic carditis), the foregoing investigations should be borne in mind when dosage for an exposure which would place the heart in the path of the irradiation is prescribed.

CONCLUSIONS

1. The precordium of forty-eight rats and twenty-four rabbits (leaving two or three in each group of twelve animals as controls) was exposed to roentgen rays (lambda-effective equaled 0.16 and 0.23

6. Hartman, F.; Bollinger, A.; Doub, H., and Smith, J.: Heart Lesions Produced by the Deep X-Ray: An Experimental and Clinical Study, Bull. Johns Hopkins Hosp. **41**:36, 1927.

7. Renfer, E.: Ueber zwei Fälle von Radiumnekrosen des Myokardes, Le cancer, 1927, Jg. 4, 431, quoted from Zentralbl. f. ges. Radiol. **4**:586, 1928.

8. Levy, R., and Golden, R.: Some Effects of Roentgen Irradiation of the Heart in Rheumatic Carditis, Am. J. Roentgenol. **18**:103, 1927.

angstrom units, respectively) three times at monthly intervals. The dose amounted to 600 R the first time and 300 R each the second and third time. The animals were killed from eight to thirty-two days following the last exposure and the hearts examined microscopically.

2. In every animal irradiated, definite lesions were found in the myocardium, consisting essentially of marked Zenker's necrosis and fatty degenerative infiltration. In many cases there was evidence of inflammatory reaction and early repair. These myocardial lesions were associated in every irradiated animal with a more or less severe bronchitis and bronchopneumonia and with other evidences of mediastinal roentgen-ray injury to the thymus, lymph nodes and ganglions. We regard the pulmonary infection as secondary to the irradiation injury of the bronchi, lowering the resistance and being followed by secondary bacterial infection. A vicious circle is therefore set up increasing the myocardial change.

3. We conclude, therefore, from these investigations that definite injury of the heart muscle results from a series of exposures as described. This suggests caution in any case in which the treatment brings the heart in the path of the rays.

4. An attempt will be made to define the threshold value of roentgen-ray energy for the lesions described, or in other words, the tolerance dose for the heart muscle.

ABSTRACT OF DISCUSSION

Dr. Frank W. Hartman, Detroit: Our work was done with the purpose of producing an experimental heart lesion. I am very glad that Dr. Warthin and Dr. Pohle have been able to confirm our results on animals. Our first work was done at about the same time that Dr. Warthin and Dr. Pohle started theirs. As to the apparatus and dosage, we used relatively large doses, but the apparatus was carefully calibrated. In fact, Dr. Pohle calibrated our apparatus. The dosage was carefully controlled—the distance, voltage, amperage and everything, except the ionization chamber which Dr. Pohle mentioned, was taken into account. The doses were unusually large because we started out to produce an experimental lesion so that we might study the irregularities in the heart following these permanent lesions. As to the experiments reported by Dr. Pohle, it seems to me that the presence of so much infection would largely obviate the possibility of definite conclusions. We used dogs rather than rats and rabbits, and in only one case did we see any evidence of infection in the heart or pericardium. This animal had a bacterial endocarditis and a septicemia. With this exception, the changes reported by us may be accepted as being due to the roentgen ray. As to cases in human beings, Dr. Warthin will possibly feel differently about them after he has reviewed the original sections made from the hearts. Many of you saw our demonstration last year in which three cases in human beings were presented. Since that time we have seen another. All of these were cases of mediastinal tumor and large doses of irradiation were given ranging from 60 to 200 minutes with the same voltage, amperage, filterage and distance used in our experimental work. I am not enough of a physicist or radiologist to tell you how that can be compared with

the dosage described by Dr. Pohle, but I do know that it is well within the limits of dosage used for malignancy of the mediastinum. If you recall some of the high power photomicrographs of the myocardium as shown in the demonstration last year you will remember that about every other muscle fiber appeared as a hollow tube the center of which was filled with large vacuoles or occasionally a single coagulated mass of protoplasm. I do not think that anybody would deny that such a lesion is produced by the roentgen ray. Similar marked changes in human beings have been described by German authors, particularly by Schweitzer.

There is another phase of the work that I should like to emphasize and that is the fallacy of applying results obtained on one animal to other animals or to man. We reported lesions produced in dogs, sheep and human beings. We found that the dogs are much more susceptible than sheep, and dogs are somewhat more susceptible than man. As Davis pointed out several years ago, the right auricle in the dog is particularly susceptible, and even with moderate doses projected through the wall of the chest, lesions are produced. Davis did this work while he was showing clinical changes in the lung. In the sheep it is entirely different. You can project as much as 300 minutes of the dosage, such as Dr. Pohle has mentioned through the anterior aspect and side of the chest in sheep, and you will not get any such hemorrhagic lesions. We do not see these hemorrhagic lesions in man either, but the sheep die just the same from myocardial degeneration and some of the most marked instances of necrosis and polymorphonuclear infiltration were shown in these sheep. There is also a large variability between individual animals. I do not think this is accounted for by difference in the contour of the chest or from difference in roentgen technic, but there is a large difference in susceptibility. I have no doubt that men with large experience have seen the same thing in human patients. One other thing is the possibility of controlling the effects in human beings with the electrocardiograph. We have been able to do that satisfactorily in our dogs and sheep, and in the last year we have been following up by means of the electrocardiograph every case in which irradiation was given over the chest. We have been able to show that when the voltage becomes low or when the T waves become inverted, the myocardium is suffering and it is time to rest a while.

DR. G. E. PFAHLER, Philadelphia: We must especially emphasize the point brought out by Dr. Hartman, namely that need for caution in transferring data obtained in the treatment of experimental work on small animals to man. Take the size of the animals. Judging by the diagrams shown by Dr. Pohle, at least three fourths of the animal's chest was exposed, and about one third of the entire body. Then we give this small animal the doses we would give to a human being! We go a step further, according to this technic, and give a full dose of roentgen rays to this animal, a massive dose, which I think none of us do at the present time; and then he repeats that in one month, and again in another month. I know of no one who repeats a massive dose inside of two months in a human case. Therefore, we must conclude that relatively this animal received at least ten times the relative dosage that would be given to a human being. The results obtained in this experimental work are inconsistent with the clinical observations all of us have made. I have treated over 1,000 patients with carcinoma of the breast which involved more or less exposure of the heart. I have never seen any clinical symptoms to indicate that the heart was affected. I have performed many autopsies and no such results have been reported as have been indicated in this experimental work. Within the past year we had a very complete autopsy in a case of Hodgkin's disease. I had treated the patient in 1920. There was a large mass about twice the size of the heart in the mediastinum.

This mass completely disappeared under radiation, and, of course, we had to treat the heart. The patient finally died of the disease, and at autopsy, Hodgkin's lesions were found in all the tissues of the body, including the heart, and yet there were no such results reported as we find in this case. The animal experiments shown had associated a pneumonitis. You can produce a pneumonitis in a human being by excessive dosage, but why should we give it?

DR. HENRY SCHMITZ, Chicago: Without entering into a discussion of the comparability of animal experimentation with our clinical observations on the human being, I have for years made the statement that when one massive treatment has been given — particularly for carcinoma — the treatment should not be repeated, because the tissue cannot stand a second full dose without permanent injury or damage. When we repeated a massive dose, within varying intervals, from six months to two years, we have regretted doing so. In each instance irreparable damage ensued from which the patient later succumbed. Let us content ourselves with one massive treatment as the surgeon is content with one operation. Recently we had an opportunity to observe a patient who was suffering from carcinoma of the breast. She had been treated elsewhere with interrupted frequent doses. We gave her a single massive treatment. After three years she developed a nodule which showed carcinoma. The treatment was repeated and before it was finished the patient became ill with an extreme disturbance of the heart, and it was six weeks before she finally recovered from the effects of this treatment.

DR. E. A. POHLE: As far as measuring of the dose of roentgen rays in Dr. Hartman's experiments is concerned, I will simply quote part of the resolution of the Standardization Committee of the Radiological Society of North America. "Voltage and filtration alone do not properly define radiation quality. It should be measured by a reliable ionization instrument." The same holds true for the quantity of radiation. As Dr. Hartman stated, "No such instrument was employed" and this should settle the argument. I was interested in listening to Dr. Pfahler's remarks. We agree as far as transferring the results of these animal experiments to human beings is concerned. In our first paper this fact was emphasized. Regarding the dose applied, there must have been a misunderstanding. We did not give three times a full dose within three months. The first time a full dose was given and the second and third time 50 per cent of it. In the old terminology it would amount to two erythema doses within twelve weeks. According to the so-called saturation method advocated by Dr. Pfahler in 1925 — such a procedure would be permissible in human therapy. He states that two full erythema doses of roentgen rays, of the penetration used in our experiments, may be given within from eight to twelve weeks. We must also consider that 600 R as adopted in our work as a tolerance dose (erythema dose) is identical with the surface dose because we used small fields. In therapy of conditions of the chest in people large fields of entry are employed adding about 40 per cent to the surface dose by back-scattering. Some laboratories use larger doses, up to 900 R measured in air, as erythema dose. A single exposure through a large field would bring the surface dose up to over 1,200 R. In our experiments this value is reached by the total dose given in divided doses over a period of three months. I do not feel, therefore, that this can be called excessive. The ratio of the irradiated volume to the total volume of the body is rather hard to establish. However, I would not be surprised if a comparison of the two ratios in human beings, using a 20 by 20 cm. field of entry, and in rats treated over the heart with our technic will be in close proximity.

THE HEART IN THYROID DISEASE

I. CHANGES IN THE T WAVE OF THE HUMAN ELECTROCARDIOGRAM FOLLOWING IODINE MEDICATION AND THYROIDECTOMY *

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Fifty years before Graves' and Basedow's description of the symptom complex bearing their respective names, Parry and Flajani called attention to the occurrence of disturbances of the heart in association with goiter.[1] Adelmann appears to have described two forms of disturbances of the heart in goiter, while Potain contributed a clinical discussion to the subject.[2] Rose first emphasized the importance of the heart in the sudden deaths of patients with goiter, believing that the effect on the heart was purely mechanical.[2] Schranz, however, and with him Wölfler and Wette, not entirely content with the mechanical theory, added reports of cases in which they believed nervous factors were also operative.[2]

In 1898, Friederich Kraus of Berlin described and differentiated various forms of goiter heart, dividing them finally into two principal groups; first, the mechanical goiter heart, and second, the monosymptomatic goiter heart.[2] The monosymptomatic goiter heart of Kraus was found in patients with goiter but without mechanical embarrassment of the circulation or respiration. Kraus believed that these cases never progressed to a true exophthalmic goiter but that they showed predominantly a cardiovascular syndrome, namely, increased cardiac activity, often tachycardia, a bounding, rapid pulse, in severe cases arrhythmia and in protracted grave cases, decompensation. Clinically and at autopsy the heart was enlarged to the left, in contrast to the mechanical form of goiter heart with enlargement of the right side.

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** From the Thyroid Group Service and the Cardiographic Laboratory, Michael Reese Hospital.

* Read before a meeting of the Association of American Physicians, Atlantic City, May 3, 1927.

1. Quoted from Klose: Die Basedowsche Krankheit, Ergebn. d. inn. Med. u. Kinderh. **10**:182, 1913.

2. Quoted from Chvostek, F.: Morbus Basedowi und die Hyperthyreosen, Berlin, 1917, p. 372.

Chvostek [3] gave the most complete description of the heart in thyroid disease, classifying the various forms as follows:

1. Mechanical goiter heart of Rose (the pneumatic or dyspneic goiter heart of Minnich).
2. The congestive goiter heart (substernal goiter) (goiter cardiaque, Revilliod).
3. Neurotic goiter heart (Wölfler) from pressure of the goiter on the vagus and sympathetic.
4. Thyreotoxic goiter heart (Kraus).
5. Degenerative or torpid goiter heart (Bauer) (myxedema?).
6. Thyreopathic cardiac hypertrophy (Minnich) (myxedema?).

In a clinical discussion of the goiter heart, Gmelin [4] called attention to certain characteristics of the tachycardia, namely, that it is constant, even with rest in bed, and at night; that the tachycardia is not influenced by drugs or narcotics, and that it disappears promptly after operation. He spoke of the steep ascent and quick descent of the pulse wave during tachycardia, which he believed is due to abnormal and persistent dilatation of small vessels. He described the heart beating violently against decreased peripheral resistance, the subjective sensation of warmth, the tendency to excessive perspiration and the profuse bleeding in the operative field during thyroidectomy, all evidence of the same capillary and arteriolar dilatation.

The present study is the first of a series of clinical and experimental observations concerning the heart in thyroid disease. While for many years this subject has been the recipient of much careful experiment and observation, there still appear to be many unsolved problems. Suggestive evidence of the desirability of further work concerning the rôle and integrity of the heart in thyroid disease may be seen from the following conflicting statements from both old and recent studies on this subject, some of them emanating from clinics where a vast amount of thyroid material is available for study:

Basedow patients suffer and die through their hearts. (*Moebius.*)

Without tachycardia no Basedow. (*Charcot.*)

There are probably no cases of exophthalmic goiter in which cardiac symptoms are completely wanting. (*A. Kocher.*[5])

From a study of the data presented in this paper, the most outstanding fact is the infrequency in both exophthalmic goiter and adenoma with hyperthyroidism of symptoms indicating cardiac disease. (*Willius, Boothby and Wilson.*[6])

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3. Chvostek, F.: Morbus Basedowi und die Hyperthyreosen, Berlin, 1917, p. 372.

4. Gmelin, E.: Zur Klinik des Kropfherzens, Arch. f. klin. Chir. **143**:726, 1926.

5. Kocher, A., in Kraus and Brugsch: Specielle Pathologie und Therapie, 1919, vol. I, p. 751.

6. Willius, F. A.; Boothby, W. M., and Wilson, L. B.: The Heart in Exophthalmic Goiter and Adenoma with Hyperthyroidism, M. Clin. N. Amer. **7**:189 (July) 1923.

Young individuals with previously undamaged hearts suffer no cardiac changes, no matter how intense the thyroid toxicity. (*Lahey and Hamilton.*[7])

It will be noted that in each of this series (hyperthyroidism) with one exception, the volume output of the heart per beat was diminished; . . . it would therefore appear that all these patients had some heart failure. (*Rabinowcitch and Bazin.*[8])

With these conflicting opinions in mind, we propose to approach the problem from a number of separate points of view, both clinical and experimental, collecting data by methods in which the subjective element is eliminated or relegated, as far as possible, to the background.

The widespread and enthusiastic reintroduction of iodine into the therapy of hyperthyroidism offers an additional reason for reopening the problem of the goiter heart. It is common experience that although in most cases and at certain stages in the evolution of exophthalmic goiter iodine has great value, in other cases and at other stages in the disease, it appears to be distinctly harmful. A number of interesting pieces of work are available in this connection. For example, in a study of the physiologic interrelations of the thyroid and heart, von Cyon [9] found that iodothyrin exerts a definite influence on the regulatory cardiac nerves, markedly increasing the normal excitability of the vagi and depressor nerves, and that it is effective in restoring the diminished excitability of these nerves as a result of thyroidectomy or degenerative disease of the thyroid gland.

Two years later, Barbera [10] confirmed von Cyon's observations, emphasizing as Boruttan had the antagonistic action of atropine and the stimulating effects on the vagi of iodine and iodothyrin.

Goodpasture [11] fed thyroid gland substance (thyroxin) to rabbits and produced areas of perivascular fibrosis and necrosis in the heart muscle. The same year, Hashimoto [12] produced experimental myocarditis in animals with toxic doses of dried thyroid substance and found interstitial tissue lesions not unlike the Aschoff-Tawara nodes in acute rheumatic fever.

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7. Lahey and Hamilton: Thyrocardiacs; Their Surgical Treatment, Surg. Gynec. Obst. **39**:10 (July) 1924.

8. Rabinowitch, I. M., and Bazin, E. V.: The Output of the Heart Per Beat in Hyperthyroidism, Arch. Int. Med. **38**:566 (Nov.) 1926.

9. Von Cyon: Zur Physiologie der Schildrüse und des Herzens, Pflügers Arch. f. d. ges. Physiol. **70**:511, 1898.

10. Barbera: Einfluss von Iodin, Iodonatriam und Iodothyrin auf der Kreislaufs, Pflügers Arch. f. d. ges. Physiol. **79**:312, 1911.

11. Goodpasture, E. W.: The Influence of Thyroid Products on the Production of Myocardial Necrosis, J. Exper. Med. **34**:407, 1921.

12. Hashimoto, H.: The Heart in Experimental Hyperthyroidism with Special Reference to Its Histology, Endocrinology **5**:579, 1921.

In an interesting study of metabolism of iodine, with particular reference to the level of iodine in the blood under various conditions, Veil and Sturm [13] found an increase of iodine (hyperiodemia) during tachycardia, and sympathetic stimulation by epinephrine and a decrease (hypo-iodemia) in vagus stimulation with pilocarpine and under digitalis medication.

Takane [14] believed that acute myocarditis, produced experimentally by inorganic iodine compounds, was caused by the liberation of free iodine and by the large amounts of lactic acid present in the actively contracting heart muscle.

A summary of these studies shows that:

Iodothyrin increases the excitability of the vagus and depressor nerves.

Thyroxin produces perivascular and interstitial fibrosis and necrosis of the heart muscle.

Sympathetic stimulation causes hyperiodemia.

Vagus stimulation causes hypo-iodemia.

Experimental iodine myocarditis develops because of the excessive amounts of lactic acid in heart muscle unbound by protein, uniting with inorganic salts and liberating free iodine.

In other words, one may conclude from these and similar studies that under certain conditions iodine exerts a positive (harmful?) effect on both the regulatory cardiac nerves and the cardiac musculature.

Bearing in mind these conflicting views concerning the frequency of the involvement of the heart in thyroid disease and of the effects of iodine and of thyroid substance on heart muscle and cardiac nerves, we decided first to direct our attention to the electrocardiographic examination in persons with thyroid disease. In this paper we will present the results of an electrocardiographic study of patients suffering with varying degrees of thyrotoxicosis, the condition at the height of the disease before treatment, the changes in the electrocardiogram occurring during iodine medication, and the condition following operation.

Hoffman [15] appears to have been the first to describe the high T wave in the electrocardiogram of patients suffering with exophthalmic goiter. In twenty-three patients with the so-called thyrotoxic heart, a high T wave was found in lead II, the height of the wave being proportional to some extent to the rapidity of the heart beat. Almost a decade later,

13. Veil, W. H., and Sturm, A.: Iodin Metabolism. Deutsches Arch. f. klin. Med. **147**:166, 1925.

14. Takane, K.: Pathobiogenese der Myocarditis acuta durch organische und anorganische Jodbindungen bzw. der Basedomyokarditis, Virchows Arch. f. path. Anat. **259**:11, 1926.

15. Hoffman, A.: Die Elektrocardiographie, Wiesbaden, 1914, p. 108.

Krumbhaar,[16] apparently unacquainted with Hoffman's work, described "unusually prominent T wave in most cases of toxic goiter, which in about half the cases were markedly and persistently diminished after operation." In a large series (377 patients) of electrocardiographic observations of the heart in exophthalmic goiter and adenoma with hyperthyroidism, Willius, Boothby and Wilson[17] refrained from commenting on the height of the T wave, except to call attention to the infrequency (1 per cent) of inversion of the T wave.

Confirmatory, though indirect, evidence of the significance of the height of the T wave in persons with hyperthyroidism is available in a number of studies of the heart in hypothyroidism. In persons with myxedema, Zondek[18] found both the P and the T waves practically absent, with a gradual return of the waves following thyroid medication. From observations in four cases, Fahr[19] believed that inversion of the T wave in lead I was characteristic of the heart in myxedema, though later it became upright under thyroid medication. From a study of a large series (168) of cases of myxedema, Willius and Haines[20] concluded that "actual myxedematous change in the myocardium explains the electrocardiographic abnormalities that disappear with treatment." The abnormalities referred to are inversion of the T wave in various leads.

PLAN OF WORK

As soon as possible after admission to the thyroid group service, patients were placed at complete rest in bed, and control readings of the basal metabolism, electrocardiograms, blood pressure determinations and distance roentgenographic examinations of the heart were made. Following the completion of this control data, compound solution of iodine (Lugol's solution) was started, 10 minims being given three times daily after meals, the foregoing examinations being repeated at frequent and stated intervals during the course of iodine medication. After varying periods of from two to three weeks, sometimes longer, patients were submitted to subtotal thyroidectomy under ethylene anesthesia. At certain intervals following operation, the foregoing data were again obtained. Thirty-two cases have been studied, and the data from them

16. Krumbhaar, E. B.: Electrocardiographic Observations in Toxic Goiter, Am. J. M. Sc. **155**:175, 1918.

17. Willius, F. A.; Boothby, W. M., and Wilson, L. B.: Heart in Exophthalmic Goiter and Adenoma with Hyperthyroidism, M. Clin. N. Amer. **7**:189 (July) 1923.

18. Zondek, A.: Herzbefunde bei endokriner Erkrankungen, Deutsche med. Wchnschr. **46**:1239, 1920.

19. Fahr, G. E.: Myxedema Heart, J. A. M. A. **84**:345 (Jan. 31) 1925.

20. Willius, F. A., and Haines, S. F.: Status of the Heart in Myxedema, Am. Heart J. **1**:67, 1925.

will be presented in collective form. Electrocardiograms and drawings enlarged to scale illustrating the most significant observations are appended.

RESULT

Tachycardia was present before treatment in practically all cases, the pulse rate being roughly proportional to the severity of the thyrotoxicosis and the level of the basal metabolism. The T wave was increased in height in several cases, but the increase was not uniform and apparently

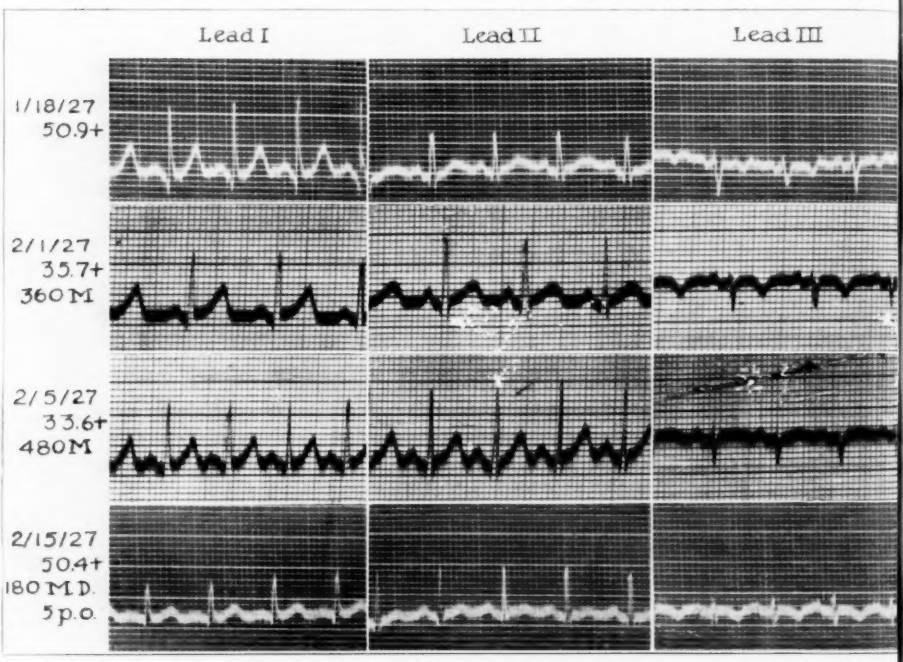

Fig. 1 (case 3).—Mildred R., a schoolgirl, aged 15 years, simple (adolescent?) goiter for past year, with sudden severe exacerbation two weeks before admission (acute exophthalmic goiter) during iodine medication. Increase of T_2 during administration of iodine with signs of decompensation. Slight improvement with digitalis. Death immediately following thyroidectomy. Pathologic examination showed hyperplasia of the thyroid with colloid invasion, focal areas of hyperplasia and moderate fibrosis.

had little if any relation to the pulse rate. Preliminary rest in bed without iodine resulted in a slight decrease in the pulse and basal metabolic rates, but was without effect on the height of the T wave. In most cases, iodine caused a varying degree of depression of the basal metabolic and

the pulse rates and in the majority of instances, a lowering of the T wave. There were, however, many exceptions to the latter, several cases not showing an apparent change. One showed a slight increase in height and one a marked increase with increase in the pulse rate and signs of decompensation. In some cases, changes in the T wave were the only ones observed; others showed a proportional decrease in the height of the P wave and still others, a decrease in the height of all waves.

The effects of thyroidectomy were more uniform. The T wave became definitely lower in most instances following operation, the degree of lowering being roughly proportional to the level of the basal metabolic and pulse rates, irrespective of the height of the wave prior to operation and iodine medication. This postoperative depression remained permanent in most instances, as long as the patient remained under observation, although two cases finally showed a gradual return to

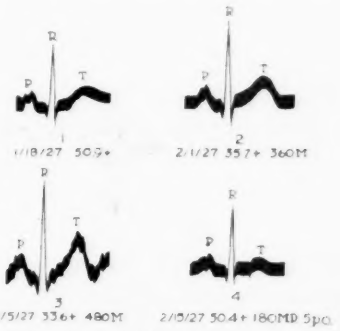

Fig. 2.—Enlarged drawing (to scale) of electrocardiographic complexes (lead II, fig. 1), showing changes in T wave during iodine medication and following thyroidectomy (4). Enlarged drawing showing (*a*) the date curve was made; (*b*) basal metabolic rate; (*c*) amounts of compound solution of iodine (Lugol's solution) administered up to that time expressed in minims.

the preoperative level. In two cases, the lowering of the wave progressed to a definite inversion, the basal metabolic rate meanwhile not showing a proportional decrease. The degree of inversion of the T wave varied at definite periods following operation with, however, a gradual return to a point just below the preoperative level.

In the two cases showing inversion of the T wave, the negativity occurred after a rather long iso-electric period from the foot of the R wave, what might be called delayed inversion. This type of inversion is in contrast to the immediate inversion of the T wave from digitalis, and the "cove"-shaped T wave of coronary occlusion.

While in most cases the changes in the T wave were most striking, several cases showed parallel changes in the other waves. In practically

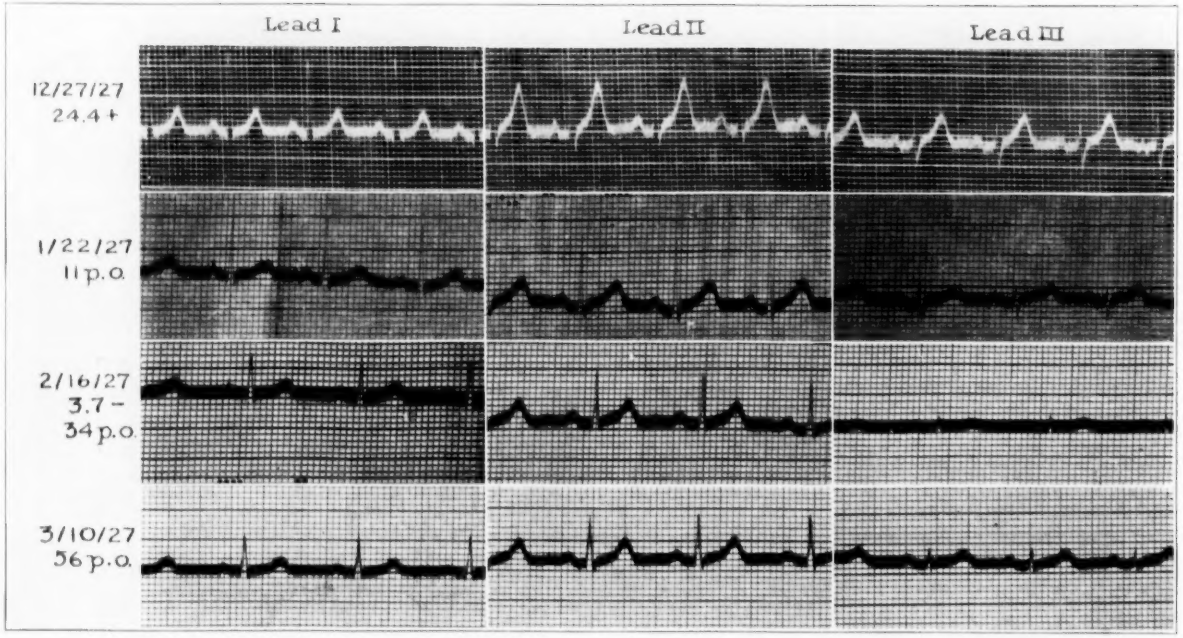

Fig. 3 (case 4).—Miss Bertha S., a school teacher, aged 47. Toxic adenoma of eight years' duration, with severe acute exacerbation of symptoms (sympathetic nervous system) for the past three to four months. Unusually high T waves occurred in all leads during administration of small amounts of iodine, with marked decrease eleven days after thyroidectomy, with still further lowering of T_3 thirty-four days after operation.

all instances, as the T wave decreased in height the R-T interval lengthened, and conversely.

In searching for an explanation of the source of these observed electrocardiographic changes, one must consider certain facts and characteristics concerning the T wave. Without attempting to discuss at length the various theories and beliefs concerning the nature of the T wave, both experimentally and clinically, all that we wish to call attention to is the generally accepted fact of the extreme lability of the T wave, a phenomenon observed and commented on by all workers in this field.[21]

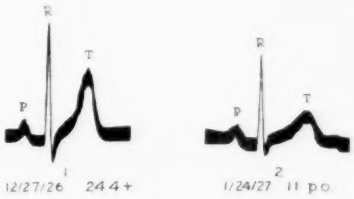

Fig. 4.—Enlarged drawing (to scale) of electrocardiographic complexes (lead II, fig. 3), showing high peaked T_2 and a basal metabolic rate of 24.4 + before treatment, with decrease in height of T_2 occurring eleven days postoperatively (subtotal thyroidectomy).

Fig. 5.—Enlarged drawing (to scale) of electrocardiographic complexes (lead III, fig. 3), showing high peaked T_3 before treatment with marked lowering (almost to iso-electric line) of T_3, thirty-four days postoperatively, with a basal metabolic rate of 3.7 per cent minus.

To quote from Lewis:[22]

It is agreed by most workers that in its direction and form, T is much less stable than are the initial (ventricular) deflections. Thus vagal or sympathetic stimulation may exert a profound influence on the end deflection though the initial deflections are affected slightly. Many poisons act similarly. As Cohn and his

21. Katz and Weinman: The Relation of the T Wave to the Asynchronism Between the Ends of Right and Left Ventricular Ejection, Am. J. Physiol. **81**:360, 1927, found "no consistent relation between changes in the T wave and variations in synchronism of the end of right and left ejection."

22. Lewis, T.: The Mechanism and Graphic Registration of the Heart Beat, ed. 3, London, Shaw & Sons, 1925, p. 120.

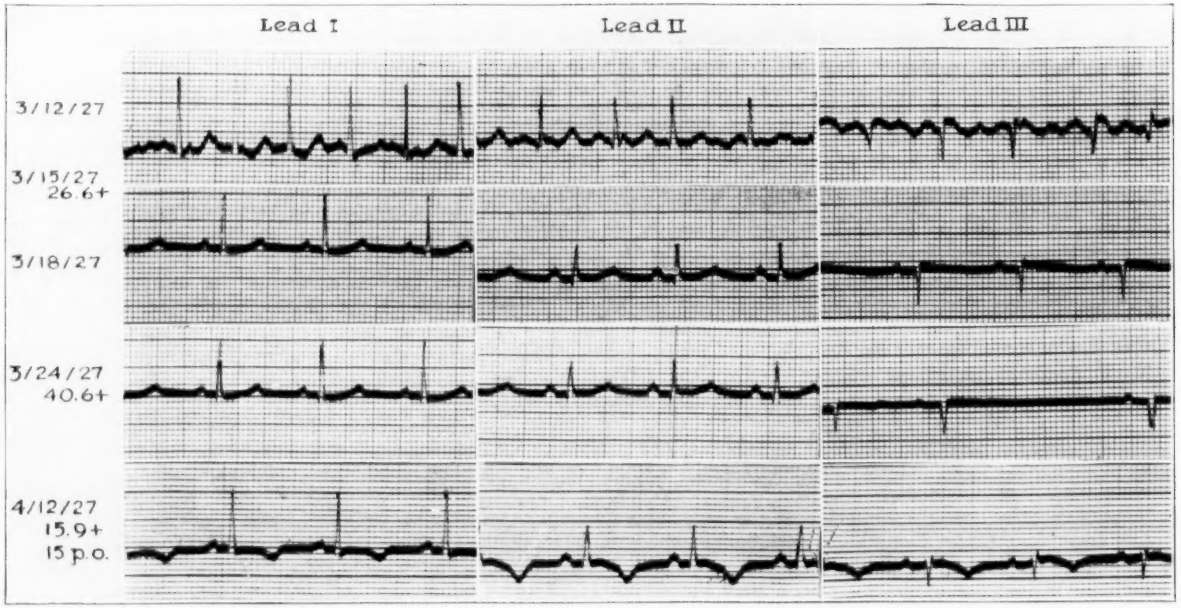

Fig. 6 (case 6).—Mrs. Anna J., housewife, aged 62. Toxic symptoms of one year's duration following influenza, with attacks of paroxysmal fibrillation and sino-auricular block. T waves low throughout—iso-electric in lead III. No change after iodine, quinidine or digitalis medication. Inversion of the T wave occurred in all leads, following thyroidectomy.

co-workers [23] have shown, an early effect of digitalis intoxication in man is to invert T deflection, QRS remaining unchanged.

The reference to vagal and sympathetic stimulation emanates largely from the experimental observations of Rothberger and Winterberg. The accompanying table (taken from Tigerstedt [24]) summarizes their most important conclusions.

Summary of Conclusions of Rothberger and Winterberg

Condition of Regulatory Nerves	P.	R.	T.
High vagal tone	low	high	low
Vagal activity (right ventricle)		high	low
Fall in accelerator tone (Stellectomy)	low	high	low
High accelerator tone	high	low	high
Stimulation right accelerator	high	low	diphasic
Stimulation left accelerator	low	(deep S)	inverted

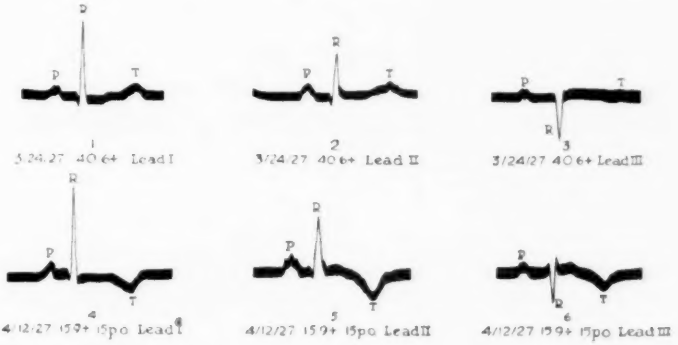

Fig. 7.—Enlarged drawing (to scale) of electrocardiographic complexes, all leads (fig. 6), showing inversion of T wave in all leads, fifteen days postoperatively (thyroidectomy).

On the basis of these results it is suggestive that the observations described, resulting from iodine medication and following thyroidectomy, are due, at least in part, to variations in vagal and sympathetic (accelerator) tone. These variations in tone are probably due, in the first instance, to the effects (hypertonicity?) of the thyrotoxicosis on the regulatory nerves; the later changes, to the modifying influence of iodine and thyroidectomy. These results bring additional evidence to support the view, long held, that increase and variation in sympathetic and vagal

23. Cohn, Fraser and Jamieson: Influence of Digitalis on the T Wave of the Human Electrocardiogram, J. Exper. Med. **21**:593, 1915.

24. Tigerstedt: Die Physiologie des Kreislaufer, Berlin and Leipzig, Walter De Gruyter & Co., 1923, vol. 2, p. 400.

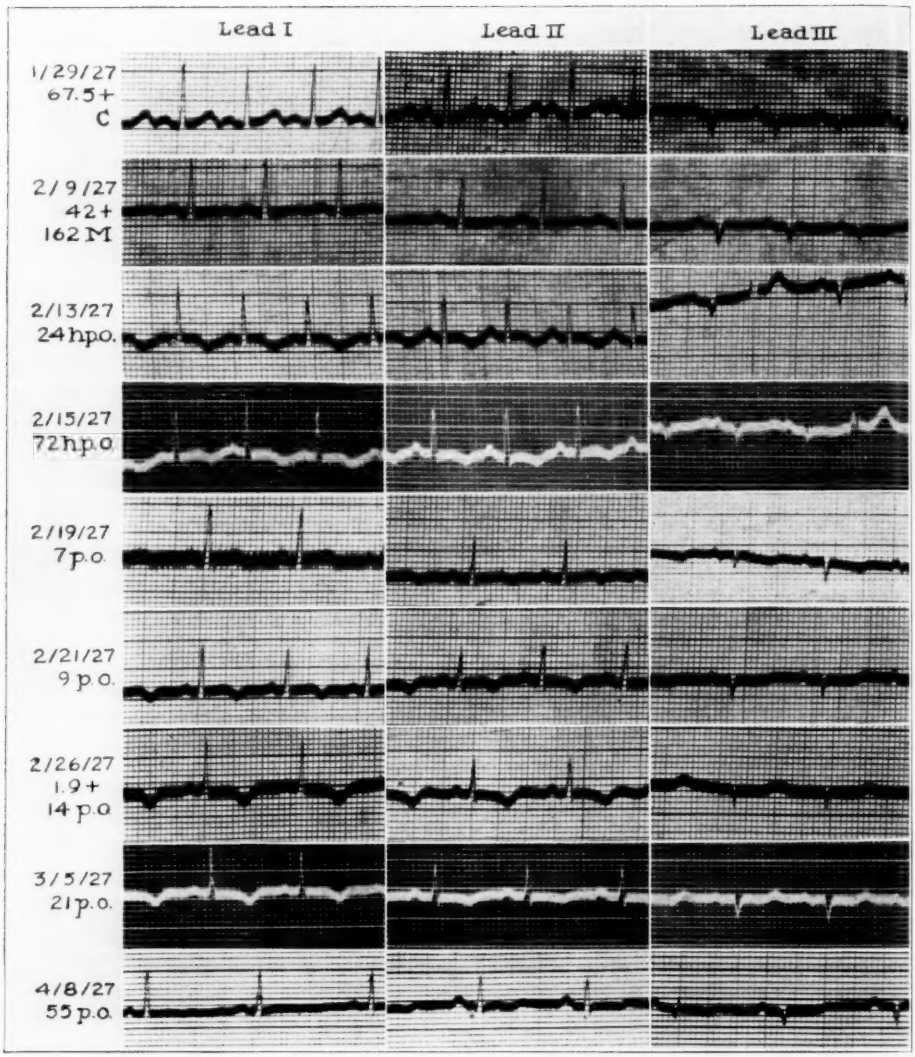

Fig. 8 (case 8).—Mrs. E. O. A., aged 38, a housewife. Toxic adenoma with sudden onset one year before presentation. Normal T waves at onset, with marked lowering during administration of compound solution of iodine (Lugol's solution), progressing to inversion immediately following thyroidectomy, inversion continuing for three weeks postoperatively, with varying degrees of negativity, becoming iso-electric and diphasic eight weeks following operation.

tone are features in the symptom complex of hyperthyroidism and probably explain the electrocardiographic changes seen during the course of medical and surgical treatment.

These results do not permit any definite conclusions to be drawn as to the effects of thyrotoxicosis on heart muscle, and while we feel that such is undoubtedly the case, objective evidence supporting this view must be sought from other sources. Whether iodine can produce similar

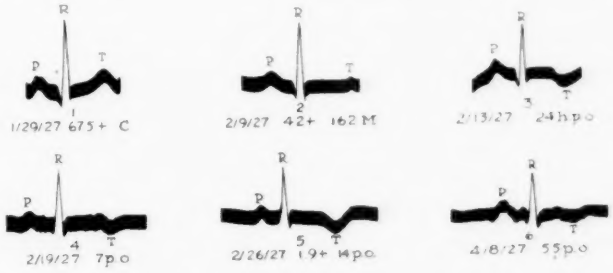

Fig. 9.—Enlarged drawing (to scale) of electrocardiographic complexes (lead II, fig. 8), showing marked lowering of T wave following administration of 162 minims of compound solution of iodine (Lugol's solution), with inversion of T wave postoperatively.

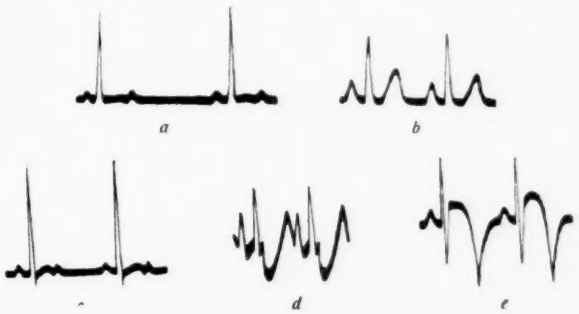

Fig. 10.—Taken from Tigerstedt (Die Physiologie des Kreislaufes, Berlin and Leipzig, Walter De Gruyter & Co., 1923, vol. 2, p. 400), showing changes in the electrocardiogram resulting from nerve stimulation. *a*, normal high vagal tone; *b*, normal good accelerator tone; *c*, effect of absence of accelerator tone; *d*, stimulation of right stellate ganglion and *e*, stimulation of left stellate ganglion.

cardiac changes in normal persons cannot be definitely answered at this time. In two control patients included in this series, the results are conflicting, one showing similar changes in the T wave, one not showing any changes (fig. 11). We plan a larger series of control cases in the near future.

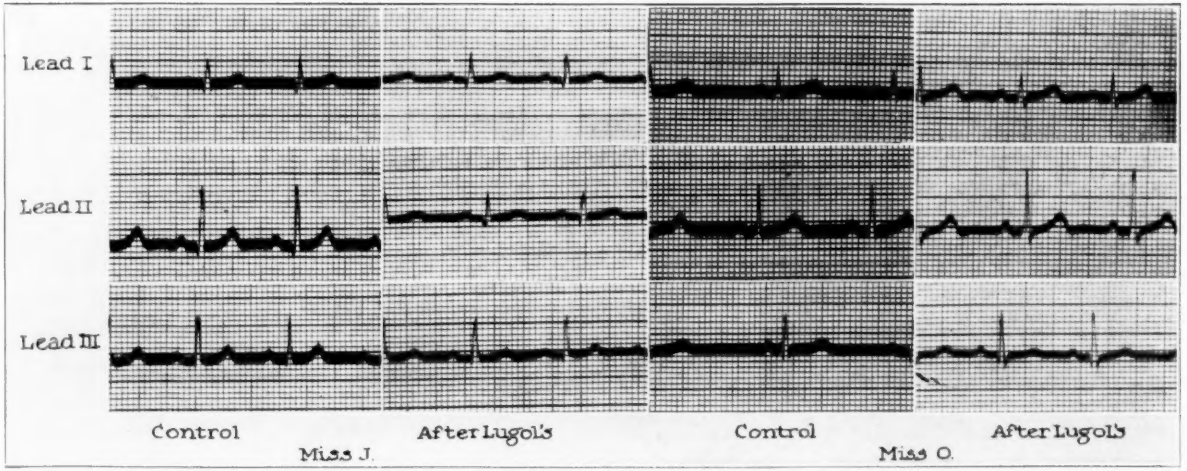

Fig. 11.—Administration of compound solution of iodine (Lugol's solution) caused slight decrease in the T wave in all leads of the first normal person (Miss J.), but no change in the second (Miss O.). The basal metabolic rate remained stationary in both.

Our observations likewise raise the question whether some part of the favorable influence of iodine medication in persons with hyperthyroidism may not be due to direct effects on the sympathetic and vagus system, independent of its effect on the thyroid, or possibly on these nerve structures through and by way of the thyroid or possibly both.

While, as we have stated, these results do not permit the drawing of definite conclusions regarding the heart muscle, it is of course obvious that a possible factor in the explanation of these electrocardiographic changes is the effect of toxic thyroid substance and iodine on heart muscle. In order to reach a definite decision regarding this, we are having recourse to direct experiment on animals as well as further work in the clinic concerning the use of barium chloride and atropine, which will be reported later.

The likelihood that diminution and inversion of the T wave may be caused, at least to some extent, by the effects of iodine on heart muscle appears likely from the effects of other drugs on the T wave, notably digitalis. If this is so, it would mean that iodine and iodothyrin may cause alterations in the muscular state of the heart. The preponderance of electrical activity persisting longer in the region of the apex than at the base, that is to say, the greater electric activity near the apex, would account for apex negativity and consequent inversion of the T wave.

CONCLUSIONS

1. Iodine medication (compound solution of iodine [Lugol's solution]) in hyperthyroidism results in most instances in diminution of the height of the T wave of the human electrocardiogram.

2. The remaining cases did not show any change in the T wave. A few showed an increase in the height of the T wave. In one of the latter, symptoms of decompensation developed coincidentally.

3. Subtotal thyroidectomy is followed by an even greater lowering of the T wave, in two cases progressing to inversion.

4. Decrease in the basal metabolic rate proceeds parallel to a decrease in the height of the T wave following iodine medication and thyroidectomy.

5. The variations in the height and form of the T wave described are probably to be explained both by variations in tone of the regulatory cardiac nerves (vagus and sympathetic) as well as by alterations in the muscular state of the heart.

6. Inversion of the T wave following thyroidectomy appears as a delayed inversion, in contrast to the immediate inversion occurring after the administration of digitalis and the cove-shaped inversion of coronary occlusion.

SPECIFIC SERUM TREATMENT IN CHRONIC ULCERATIVE COLITIS*

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ROCHESTER, MINN.

Vaccine prepared from the diplostreptococcus, which I isolated in 1924 [1] and which appears to have etiologic significance, has been shown to be of value in the treatment of patients with chronic ulcerative colitis. Various reports have appeared in the literature on the results of treatment in isolated cases, and the treatment has been used in a considerable number of unselected cases in the Mayo Clinic. The treatment has been most successful in cases of chronic invalidism in which the temperature is normal or nearly normal, the secondary anemia is only moderate and the disease has run a progressive downward course, yet apparently leaving in the patient a fighting reserve. The vaccine apparently has little value in the small group of cases in which symptoms of the disease develop rapidly as an acute fulminating illness, with fever, leukocytosis, abdominal cramps, tenesmus, many bloody, purulent rectal evacuations and rapid wasting. In these cases the patient's protective forces are taxed to the utmost by the infection, and further call on the mechanism of antibody formation goes unanswered.

The first attack of acute ulcerative colitis or acute exacerbation of chronic ulcerative colitis is striking and characteristic. The patient is acutely ill, the fever is septic, the abdomen is diffusely tender, there may be cold perspiration, the facies is frequently anxious and severe cramps may accompany the frequent rectal discharges. Often the patient complains bitterly of soreness and tenderness of the anus. The proctoscopic picture is pathognomonic, and an experienced proctologist does not confuse it with that of any other rectal disease. This is the stage of intense edema and hyperemia with myriads of miliary abscesses studding the mucosa, some of which have broken down to form minute ulcers. By carefully cleansing and, as nearly as possible, sterilizing the rectal mucosa through an anoscope, the tip of a minute Pasteur pipet may be inserted into any of the minute abscesses and a tiny drop of pus withdrawn which contains the diplostreptococcus of chronic ulcerative colitis [2] (fig. 1).

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* Submitted for publication, July 26, 1928.

* From the Division of Medicine, The Mayo Clinic.

1. Bargen, J. A.: Experimental Studies on the Etiology of Chronic Ulcerative Colitis: Preliminary Report, J. A. M. A. **83**:332 (Aug. 2) 1924.

2. Bargen, J. A.: The Medical Management of Chronic Ulcerative Colitis; A Statistical Study of 200 Cases, Tr. Am. Gastro-Enterol. A., 1927, p. 145.

Roentgenograms by barium enema also present characteristic signs. Intense spasm permits barium to enter the colon only with difficulty and causes its rapid expulsion (fig. 2).

Passive immunization of a patient with such a fulminating infection against the causative organism seems rational. With this idea in mind and for the purpose of producing a serum of diagnostic value, an effort was made to secure an immune serum in horses. Rosenow prepared the serum by injecting two horses with increasing doses of freshly isolated strains of the diplostreptococcus and by using several of these after preservation in dense suspensions in a mixture of two parts of glycerine

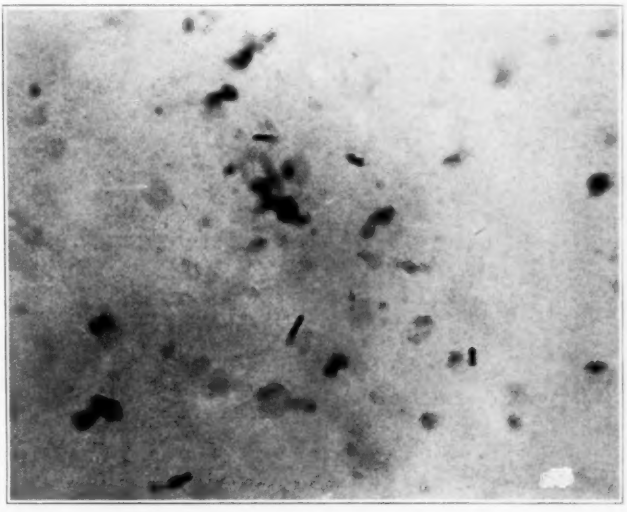

Fig. 1.—The diplostreptococcus of chronic ulcerative colitis; × 1,000.

and one part of saturated sodium chloride solution during the period of immunization (fourteen and nine months, respectively). This method has been shown to preserve specificity of streptococci for a long time, and for this reason Rosenow has used it in the preparation of poliomyelitis, encephalitis and other antistreptococcus serums. The serum was not used for treatment until after immunization had been continued for five months, and until it had a high agglutinating and precipitating titer, as well as protective power against the experimental disease in rabbits.[3] Intravenous injections were given on three successive days each week, beginning with twenty-five billion dead organisms. The dosage was

3. Results along these lines to be published elsewhere.

gradually increased to two thousand billion organisms. After immunization had been continued for two and four months, respectively, live organisms were given. Bleedings were made from seven to ten days after the last previous injection, at intervals of from three to six weeks,

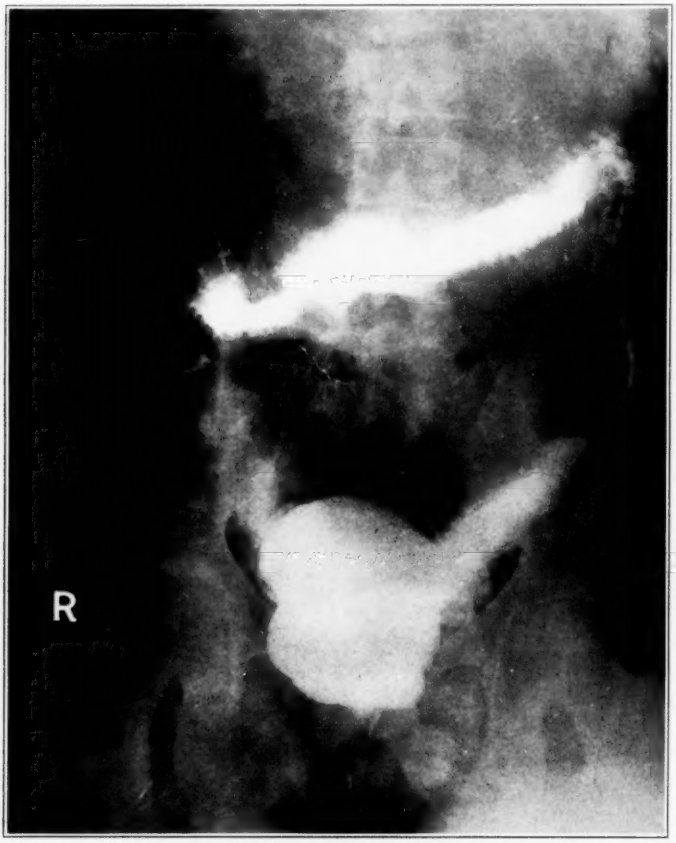

Fig. 2.—Typical roentgenogram by barium enema in a case of acute ulcerative colitis of the chronic ulcerative colitis type.

depending on the condition of the animal. The bleedings were made in a sterile manner from the jugular vein. The serum was drawn from the clotted blood after squeezing the clot with tinned weights for from twenty-four to forty-eight hours. Two-tenths per cent cresol was added as a preservative. It was proved sterile by making aerobic partial

tension and anaerobic cultures[4] and proved nontoxic by intravenous injection into guinea-pigs.

In eleven cases, deep muscular injections of the serum were given in repeated daily increasing doses over a period of from three to six days. In eight, what was considered an adequate amount was given. Four subjects were not suitable for treatment by serum; the condition in these cases was chronic, and the patients had not responded to other means of treatment. In the seven acute cases in which adequate amounts of serum were given, two patients were clinically well and have been so for seven and twelve months, respectively. The other five patients were permanently improved, the fever subsided, the blood in the stool decreased or stopped and movements were reduced to a minimum, but none was entirely relieved from symptoms. Three patients were able to carry on their business. Two patients died; one of these was improved by the serum but died at his home several months later from advancing chronic disease; the other died after ileosigmoidostomy for a suspected malignant condition; the disease had spread to the ileum and terminated finally in multiple perforations and peritonitis.

It should be noted that the condition in all seven cases was an acute fulminating ulcerative colitis. A few years ago ileostomy was performed in such cases with a high mortality rate as the result. In at least six of the seven cases, ileostomy was not necessary because the serum was used. In the present state of knowledge ileostomy must still be performed in certain cases, but it is not always easy to decide the proper time for performing it. If the patients survive the operation, the ileostomy may be permanent. Since the use of vaccine prepared from the diplococcus of chronic ulcerative colitis and the use of the specific immune serum, ileostomy has become limited to a few advanced cases in which reserve apparently has been so depleted and in which there is so much destruction of tissue that antibodies cannot develop apace with infection, and to cases with such complications as malignant growth, stricture and perforation.

Early diagnosis of chronic ulcerative colitis is imperative. A careful proctoscopic examination should be made by a competent proctologist in all cases of diarrhea. Removal of foci of infection and the administration of vaccine or serum in the proper cases promises a control of this dreaded disease.

REPORT OF CASES

Case 1.—A man, aged 52, a dealer in coal and ice, came to the Mayo Clinic on Nov. 15, 1926, with a history of bloody diarrhea (discharges about every hour) with general abdominal cramping of six weeks' duration. The leukocytes num-

4. Rosenow, E. C.: Studies on Elective Localization: Focal Infection, with Special Reference to Oral Sepsis, J. Dent. Research **1**:205, 1919.

bered 26,000. He had lost 40 pounds (18.1 Kg.) in weight in six weeks. The temperature was from 99.5 to 100 F. The Wassermann reaction of the blood was negative. Gastric acids were 60 and 48. The urine was normal. Sputum and smears from the rectal discharge did not disclose acid-fast bacilli. Parasites or ova were not found in the stool. The tonsils were infected, and one tooth contained periapical abscess. The proctoscopic picture was that of rather active chronic ulcerative colitis; the rectal mucosa was studded with innumerable small ulcers, and the mucosa bled easily. Roentgenograms of the colon were negative.

The patient was given injections of filtrate prepared from the diplococcus of chronic ulcerative colitis in increasing doses every third or fourth day. He also received tincture of iodine, 10 drops to a dose, for a few days. He was dismissed on December 10, when one or two formed stools were being passed daily. He was instructed to continue the treatment by filtrate at home and to have his tonsils removed. On February 5, he reported by letter that he had a slight exacerbation of trouble; this grew worse gradually, and he returned to the clinic on February 12. About thirty bloody, purulent stools were being passed every twenty-four hours.

On February 14, the proctoscopic picture was that of acute severe exacerbation of the disease. From February 15 to 19 inclusive, 15, 20, 30, 33 and 50 cc. of concentrated immune serum of chronic ulcerative colitis was given. On February 21, following a three-day gradual decrease in stools, one formed stool was passed. The proctoscope on February 22 showed the mucosa of the sigmoid to be normal and that of the rectum greatly improved. The patient was dismissed from observation on February 26 clinically well, and was given an autogenous filtrate to take at home. He remained well and returned for tonsillectomy on April 22. An eighteen hour culture of the macerated tonsils was injected into two rabbits; one rabbit in which lesions were not demonstrable elsewhere in the body, died in forty-eight hours from acute ulcerative colitis; the other rabbit suffered from diarrhea but recovered. The proctoscopic examination on April 28 showed the mucosa of the bowel to be entirely normal with only a tendency to bleed on trauma. From February 22 to April 22, the patient gained 22 pounds (10 Kg.). He was instructed to continue treatment by filtrate for several months.

In a letter written on October 10, the patient stated that he was still well. The serum reaction came three weeks after the first injection of serum as severe urticaria. Except for one slight upset, which lasted two weeks, and which was promptly controlled by vaccine, this patient has not had any diseased condition of the bowels for a year.

Case 2.—A woman, aged 27, a farmer's wife, came to the Mayo Clinic on March 15, 1927, complaining of bloody diarrhea of five months' duration. This condition had started with ten to twelve soft bowel movements daily, the stools containing mucus, pus and blood. On admission she felt weak and had a poor appetite. The temperature was 99 F. at 2:30 p. m. The tonsils were infected, but the results of the examination otherwise were negative. The hemoglobin content was 65 per cent, the Wassermann reaction of the blood and tests of the urine were negative. In three stools examined on three successive mornings, parasites or ova were not found. Roentgenograms showed that the teeth were normal. A roentgenogram of the colon showed that the disease involved the colon to the splenic flexure. The proctoscopic picture showed marked diffuse hyperemia and edema, with innumerable minute ulcers, graded 2. At this time the patient was passing from twelve to fifteen rectal discharges daily.

The patient was hospitalized and given five deep muscular injections of the concentrated immune serum of chronic ulcerative colitis, that is, 15, 20, 30, 40 and 50 cc. over a period of five days. She had serum sickness with marked urticaria

and pains in the joints, beginning three days after the last injection, which lasted five days. Three days after the last injection of serum, the rectal discharges averaged two daily; there was no trace of blood. She was dismissed and urged to have her tonsils removed after three weeks. She was free from symptoms until June 1, at which time mild symptoms gradually returned. The tonsils had not been removed. An autogenous vaccine prepared from the diplococcus isolated from the ulcers in the rectum was given. After fifteen injections the symptoms subsided. The vaccine was continued and on September 27, the patient again reported that she was free from symptoms. The tonsils have not been removed. There has not been a recurrence for seven months, and the patient is clinically well.

CASE 3.—A man, aged 23, came to the Mayo Clinic on Dec. 29, 1926, complaining of bloody, purulent diarrhea of seven months' duration. He was brought on a stretcher and was extremely emaciated. He had lost 35 pounds (15.9 Kg.) within six months. There were from ten to fifteen daily rectal discharges—he also suffered from acute polyarthritis.

Examination revealed the patient to be extremely ill, weak and anemic, with severe perianal ulceration. The hemoglobin was 50 per cent. Results of urinalysis were negative. Gastric analysis showed total acid 24 and free acid 10. A culture of the blood was negative on Jan. 3 and 11, 1927. Roentgenograms showed the teeth, chest, kidneys, ureters and bladder to be normal. There was a small tonsillar tag. A roentgenogram of the colon showed a lesion of the entire descending colon suggestive of polyposis. Results of examination of eight successive stools on as many mornings were negative for parasites and ova. Culture of the rectal ulcers revealed nearly a pure culture of diplococci of the morphology of those found in chronic ulcerative colitis. The proctoscope above the extensive anal ulceration showed normal mucosa up to the lower part of the sigmoid, where the picture was that of acute ulcerative colitis. Prostatitis graded 4 was present.

For several months attempts were made to clear up the perianal ulceration with local applications and various systemic medications, including cod liver oil, Blaud's pills, tincture of iodine, morphine, bismuth, gentian violet and an autogenous vaccine; when these failed, the patient was given deep muscular injections of 15, 20, 20, 20 and 30 cc. of the immune serum of chronic ulcerative colitis from April 7 to 11, inclusive. From December until April, the temperature ranged from 99 F. to 102 F. daily. The immediate reaction from the serum was the slight daily elevation of temperature, from 102 to 103 F. Three days after the last injection of serum was given, the temperature receded to a maximum of 99 F. and was usually normal for four days. On April 17, the temperature went to a maximum of 101 F. and reached this point almost daily for several weeks. There was gradual improvement, and the patient was finally dismissed on May 2, in fairly good health.

Sickness from the serum, as marked urticaria, began two days after the last injection of serum was given, and lasted for four days. The patient was given a filtrate to be administered subcutaneously at home. He returned August 5, definitely improved and weighing 136 pounds (61.7 Kg.), a gain of 9 pounds (4.1Kg.) since his dismissal. During the months he was at home he was up and about daily. He returned on October 4, with a history of marked general improvement but severe abdominal cramps in the upper part of the abdomen preceding each bowel movement, of which he averaged two in twenty-four hours. The average during the severe illness was from ten to fifteen. A roentgenogram of the colon at this time showed a filling defect at the hepatic flexure, which was thought to be malignant.

Exploration was carried out on October 13. An ileosigmoidostomy was made, with the idea of performing hemicolectomy later. The surgeon believed that the

growth was malignant. Grossly, there was the typical picture of healing following chronic ulcerative colitis of the descending colon, which was narrowed and fibrous. Following ileosigmoidostomy, symptoms of colitis recurred. The patient died five weeks later of multiple perforations of the small intestine, the colitis having extended since the ileosigmoidostomy up the ileum to involve about half of the ileum and parts of the jejunum. There were several small acute ulcers in the stomach.

Case 4.—The patient was a woman, aged 25, whose trouble had begun insidiously one year previously. During the first six months, defecation took the form of a discharge of bloody, purulent material; the number of stools averaged six daily. Then the number increased to an average of fourteen, with more bright red blood and mucus and pus. That number had not increased by the time of admission. The patient had lost 48 pounds (21.8 Kg.) in weight during the two years.

On admission, Aug. 24, 1927, the patient complained of cramps in the lower part of the abdomen and a griping pain before defecation. The temperature was 99.6 F., and the pulse rate 120. A roentgenogram showed a periapical abscess.

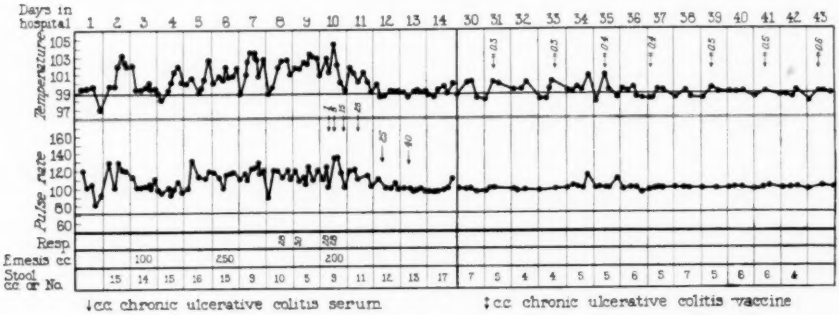

Fig. 3 (case 4).—Portion of the hospital chart, showing temperature and pulse curve, decrease in evacuations from the bowel and gain in weight following the use of immune serum.

The leukocytes on two occasions numbered 10,400 and 14,200. The proctoscopic examination resulted in a diagnosis of chronic ulcerative colitis, graded 2+. The wall of the bowel showed uniform involvement, with contraction to about a third of its normal diameter. The roentgenogram of the colon by barium enema showed that the colon was narrowed and nonhaustrated up to the splenic flexure, indicating that the disease involved at least that portion of the large bowel from the anus to the splenic flexure.

The patient appeared ill and was admitted to the hospital where she remained for thirty-six days. The rectal discharges ranged between fourteen and twenty-four in twenty-four hours. She improved gradually and was dismissed October 5; she had gained weight and was feeling better. The discharge had decreased in frequency.

After a week at a boarding house she contracted acute pharyngitis and rhinitis, with fever, followed by severe abdominal distress and a great deal more blood in the stools. She was again admitted to the hospital and during the next ten days was extremely ill (fig. 3). The number of discharges from the bowel increased,

abdominal pains were severe, and the fever ranged between 102 and 104.5 F. daily. The hemoglobin was 55 per cent; erythrocytes numbered 3,330,000 and leukocytes on two successive counts numbered 15,200 and 22,900, with 79 and 71 per cent polymorphonuclear neutrophils. Results of a roentgen-ray examination of the chest were negative, as were those of repeated urinalyses. The Widal test was negative. Repeated tests of the stools for acid-fast bacilli were negative. Nothing unusual was observed except the grave illness and the acutely tender, flaccid abdomen. Ileostomy was considered on the ninth day after admission, but the idea was abandoned.

On the tenth day a positive blood culture of the diplococcus of chronic ulcerative colitis was obtained, and serum treatment was begun immediately. The first injection consisted of 1 cc. given intracutaneously as a desensitizing dose. Two hours later, 5 cc. was injected deeply into the muscle; six hours later, 15 cc.; twenty-four hours later, 25 cc.; twenty-four hours later, 28 cc., and twenty-four hours later, 40 cc. Twenty-four hours after injections on the first day, the temperature dropped to normal and during the following two weeks did not rise above 100.5 F.; the last six days in the hospital the temperature was about normal. The discharges from the bowel gradually decreased so that on dismissal, November 13, they averaged four in twenty-four hours. The rectal discharge had not contained blood for ten days. The last ten days the patient gained 7.5 pounds (2.9 Kg.) and was feeling well.

Nine days after the last injection of serum, 0.1 cc. of an autogenous sensitized vaccine prepared from the diplococcus isolated from the patient's blood was given subcutaneously, and thereafter until dismissal an injection was given every third day. On November 28, the tooth with a periapical abscess was removed with aseptic precautions. Culture of the abscess yielded a diplococcus morphologically and biologically like that obtained from the blood during the severe illness. When this was injected intravenously into two rabbits, acute ulcerative colitis developed in one and marked diarrhea in the other.

Besides the treatment with immune serum during the acute exacerbation of the chronic ulcerative colitis and with sensitized vaccine later, a focus of infection was removed, and a high-caloric, low-residue diet was prescribed, which was gradually increased to a liberal, nourishing diet.

Case 5.—A man, aged 59, came to the Mayo Clinic on April 2, 1926, with a history of a duodenal ulcer dating back to 1916 or 1917, which had been fairly well controlled by medical treatment. Three and a half months previously, he had begun to have watery diarrhea which had gradually changed to passages of mucus and pus, a maximum of eight passages in twenty-four hours. He had lost 30 pounds (13.6 Kg.) in weight. The blood counts were essentially normal except for 5,000 leukocytes, of which 48.5 per cent were neutrophils. Gastric analysis after a test meal showed total acid 60 and free acid 48; the amount was 30 cc. The Wassermann reaction of the blood was negative. Results of urinalysis were negative. Acid-fast bacilli were not found in the stools or smears of the rectal ulceration. In three tests of the stools, on as many mornings, parasites or ova were not found. A dead tooth was found, and the tonsils were slightly enlarged. Proctoscopic examination showed the typical picture of chronic ulcerative colitis. A roentgenogram of the stomach showed a duodenal ulcer. A roentgenogram of the colon showed that the process involved the entire colon. Repeated attempts failed to isolate the diplococcus through the proctoscope.

The dead tooth was removed on April 13, and tonsillectomy was performed on April 26. A diplococcus was isolated of the morphology of those usually found in the rectum, which was agglutinated by the horse serum, and a vaccine was

prepared. The patient was given an autogenous filtrate, and he gradually improved. Alternating with the autogenous vaccine, he was given a mixed filtrate so that when he was dismissed June 10, 1926, he was getting 1.5 cc. of each alternately. He also received tincture of iodine, 10 drops to a dose, one week in every three, and kaolin as necessary. He continued treatment with vaccine at home and gradually improved until about the middle of November, when he became worse and was given four injections of neoarsphenamine (0.9 dg.). After the second injection, he was definitely better, but from then on he became steadily worse. The injections were given once a week. By the end of February, 1927, bloody stools averaged from twenty to twenty-five in twenty-four hours, and these were accompanied by fever and all the symptoms of a severe acute exacerbation of chronic ulcerative colitis. The proctoscope confirmed this. At this time the usual diplococcus was isolated which was agglutinated in high dilutions by the serum of the two horses immunized. He was given 1 cc. of the immune serum (concentrated) intracutaneously and two hours later 10 cc. On the four successive days, he was given 20, 30, 40 and 50 cc. of the serum deep in the muscle. Within a week the number of stools had reduced to eight or ten in twenty-four hours, and the blood gradually stopped. Then he had a severe serum sickness in the form of urticaria, which lasted ten days. A fresh vaccine was prepared from the organism isolated from the rectal ulcers, and this was given in gradually increasing doses. The proctoscope on April 14 showed activity graded 2 +. The patient gradually improved, receiving, besides the vaccine, gentian violet in tablet form, alternating with tincture of iodine and bismuth subnitrate as necessary. He was dismissed on July 25; the average number of stools was about six a day and he had gained considerably in weight. In a general way he was feeling much improved. In a letter written on October 1, he stated that he was still slowly improving. The form of treatment after his dismissal consisted of administration of the autogenous vaccine at regular intervals, alternating with gentian violet and tincture of iodine.

CASE 6.—A woman, aged 21, came to the Mayo Clinic on Dec. 10, 1924, with a history of abdominal pain of five months' duration, so severe that it doubled her up. The pain was followed by loose stools mixed with bright red blood. There were between six and seven discharges during the day and several at night. In the five months, she thought that there was blood in every stool. The pain was constant and described as tearing, with griping when stools were passed, and was worse after meals. There had not been fever or chills. She had lost 10 pounds (4.5 Kg.). The symptoms had grown worse during the last three weeks. On admission the blood count and the urine were essentially normal. The Wassermann reaction of the blood was negative. The proctoscope showed ulcerative colitis, beginning in the sigmoid, the first 20 cm. of the rectum being essentially normal. A roentgenogram of the colon showed a lesion of the colon, from the cecum to the sigmoid, and chronic ulcerative colitis was diagnosed. Because of the possibility of tuberculosis, exploration was advised. Acid-fast bacilli were not found in the stools, and a culture was not made.

On Dec. 29, 1924, ileostomy was performed for chronic ulcerative colitis; it was suggested that ileosigmoidostomy might be performed later, as the sigmoid seemed normal. The patient gradually recovered, went home and was fairly well until June, 1925. On Sept. 1, 1925, she returned with a history of gradual failure and four severe rectal hemorrhages, four days previously. The hemoglobin at this time was 60 per cent. The roentgenologic diagnosis was chronic ulcerative colitis. The proctoscope showed the sigmoid full of thick, bloody pus. Colectomy was performed on September 3; the pathologic diagnosis was polyposis of the

cecum, ascending colon and part of the transverse colon. An abscess developed later in the abdominal cavity and was drained on October 7. The patient was dismissed on December 16 improving, and was given some vaccine to take at home. She returned on Oct. 19, 1926, at which time the proctoscopic picture was that of chronic ulcerative colitis, the activity of which was graded 1 +. She was sent home again and given more vaccine. She returned Feb. 15, 1927, at which time the mucosa seemed to be in good condition, and she stated that she had not had symptoms for months. There was no discharge from the rectum except by irrigation (which she carried out from one to three times a week). She had gained 40 pounds (18.1 Kg.) in the year since the last operation. Ileosigmoidostomy was performed on Feb. 24, 1927. She left the clinic March 22 in splendid condition. She returned eight days later suffering from an acute exacerbation of the colitis which included the rectum and sigmoid, and extended into the ileum, and during the following five days was given 15, 20, 30, 30 and 40 cc. of the immune serum of chronic ulcerative colitis. Before this her temperature had ranged between 101 and 102 F., and there were many bloody rectal discharges. There was a

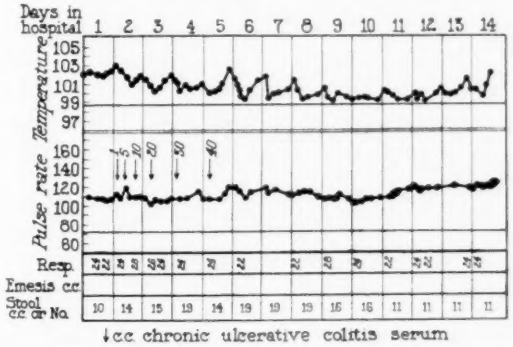

Fig. 4 (case 7).—Portion of hospital chart.

definite decrease in the amount of blood in the stools and in the number of stools after the serum was administered, and improvement continued for a week. From April 4 to 12, she suffered from a severe serum sickness, with temperature as high as 102 F., accompanied by severe urticaria. She gradually improved until September 1; from this time on, however, failure was progressive and she died September 21. This case is another illustration of the futility of ileosigmoidostomy for chronic ulcerative colitis.

Case 7.—A woman, age 37, was brought to the hospital in an ambulance Dec. 29, 1927, with a history of bloody diarrhea of ten years' duration. During the four months previous to admission, the condition had been unusually severe, confining her to bed. She had lost 13 pounds (5.9 Kg.) in four months. Rectal discharges occurred three or four times every hour and could not be controlled. The proctoscopic examination revealed the usual picture of chronic ulcerative colitis. A roentgenogram showed involvement of the entire colon. The temperature was 103 F. The hemoglobin was 56 per cent; the erythrocytes numbered 3,540,000 and the leukocytes 9,900.

From January 1 to 4, the patient (fig. 4) was given successively 1, 5, 10, 20, 30 and 40 cc. of the immune serum of chronic ulcerative colitis. The first three injections were given on the first day; the first intracutaneously, the others deep muscularly. By January 8, the temperature had dropped to 99.5 F. and varied

between 99 and 100 F. until a transfusion was given on January 13. Several days after the transfusion the symptoms again receded. The number of stools dropped to an average of ten in twenty-four hours until January 27, when a subacute perforation developed. The course was then stormy for ten days. At the end of this time the temperature receded. On February 15, vaccine treatment was begun. The patient was dismissed on March 18; the temperature had been normal for a week; there were seven stools in twenty-four hours, and she was gaining weight and was generally improved. Further treatment consisted of the use of vaccine and a nourishing, high-caloric and high-vitamin diet; improvement has been progressive.

COMMENT

In all cases in which the serum was given, there was definite improvement in the general condition. The increase in weight soon after institution of treatment with serum is significant. The serum seemed to have definite neutralizing power of the toxin of the organism causing chronic ulcerative colitis. The effect on the temperature and pulse curves is noteworthy. The outstanding improvement as shown by the proctoscope and by roentgenograms of the colon by barium enema, is of the greatest significance.

The immunization of horses is being continued. Better results are anticipated for the future. The results in this small series of almost hopeless cases are encouraging. They suggest that the present method of treatment is in the right general direction. At least two of these cases illustrate the futility and impracticability of ileosigmoidostomy in the treatment of patients with chronic ulcerative colitis.

THE RELATION OF THE HIATUS ESOPHAGEUS OF THE DIAPHRAGM TO THE STOMACH

AN IMPORTANT FUNCTION OF THE PILLARS OF THE DIAPHRAGM *

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The most commonly recognized function of the diaphragm is the part it plays in the mechanism of inspiration and expiration. The diaphragm also acts as a dividing membrane that forms the floor of the chest cavity and the roof of the abdominal cavity. When in tone, the diaphragm prevents the abdominal viscera from being pushed into the thoracic cavity. It also acts as a pressor muscle during the acts of defecation and parturition.

The function that has been least emphasized and only recently recognized is the constrictor action of the muscle at the gastro-esophageal junction. It was first described by Chevalier Jackson, and was called by him and his associates [1] a pinch cock effect. I [2] first encountered this phenomenon about four years ago during the course of certain experiments on the lung by using the transdiaphragmatic extraperitoneal route. Under certain conditions I encountered an acute distention of the stomach which caused a prompt death to the animal used in the experiment. Such a dilatation was frequently encountered during an operation on the lung when there was an undue amount of unnecessary manipulating of the lung. I also noticed a dilatation in cases in which there was rough manipulation and an excessive amount of downward retraction of the diaphragm; in cases in which an operation was performed on the lung with a preliminary resection of the phrenic nerve at the beginning of the operation, and finally in a few cases of prolonged deep anesthesia when a positive pressure artificial respiration was used with or without an open pneumothorax. Whenever the stomach became acutely distended, air could easily be pumped into the stomach, but it was difficult to remove this air even after a great amount of pressure was exerted over the walls of the stomach. Little if any air escaped from either the pyloric or the cardiac end with ordinary squeezing of the walls of the stomach. If, on the other hand,

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1. Tucker, G.: Diaphragmatic Pinch Cock in Health and Disease, M. Clin. N. Amer. **8**:931, 1924.

2. Joannides, M.: An Extraperitoneal Intrapleural Route of Approach for Intrathoracic Surgery, Ann. Surg. **80**:908, 1924; **84**:337, 1926.

a tube was inserted into the stomach, the air would escape easily and promptly. I decided, therefore, to study this phenomenon rather carefully.

In my study of the anatomy of this region, I was disappointed because I found that the descriptions of the relation of the hiatus to the esophagus were entirely different from what I found this relationship to be in the dog. Believing that there may be variations in the anatomy of the dog from that of man, I studied this relationship also in the human being and found that with few exceptions my observations on the dog were similar to those on the human cadaver. In the illustrations in

Fig. 1.—Dissection of the pillars, showing the relation of the fibers to the stomach.

textbooks, the hiatus esophageus is ordinarily so placed that one notices at least 1 cm. of the esophagus below the diaphragm. In my study of this relationship in the dog as well as in the cadaver I found that the hiatus esophageus is located at the level of the gastro-esophageal junction. I also have noticed that in the dog the relationship between the fibers of the pillars and those of the gastro-esophageal area is much more intimate than that in the human being. In the latter there is usually a layer of fibrous tissue that separates the fibers of the pillars from the stomach and esophagus. On the other hand, in the dog this fibrous layer is extremely thin so that one receives the impression that the fibers of the pillars are in apposition with those of the stomach

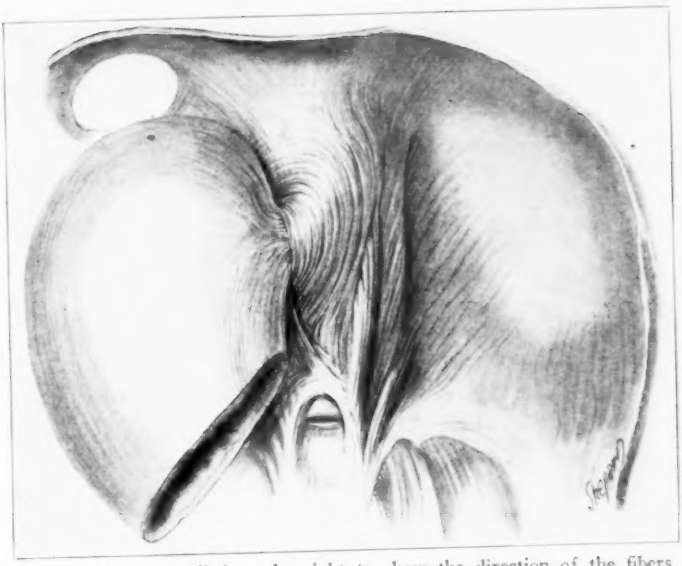

Fig. 2.—Stomach pulled to the right to show the direction of the fibers.

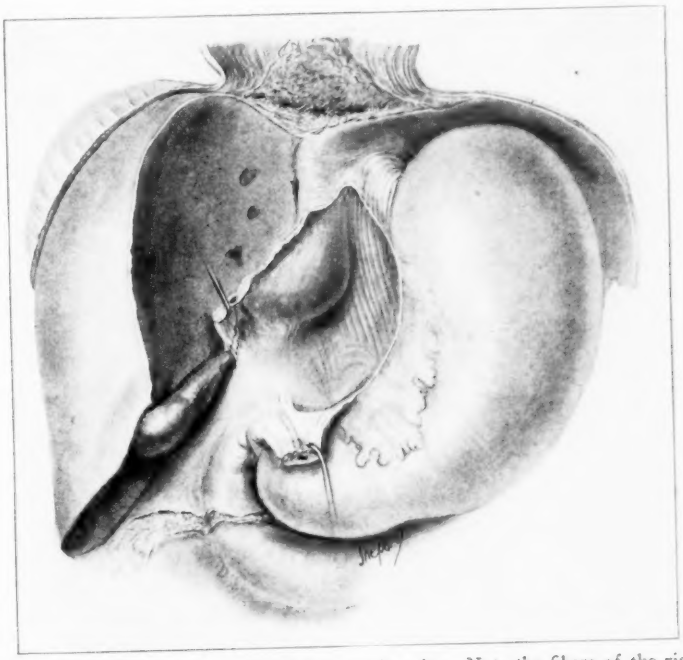

Fig. 3.—Dissection of the lesser peritoneal cavity. Note the fibers of the right pillar as they show through the peritoneum.

(figs. 1, 2 and 3). Anteriorly the fibers of the pillars surround the stomach and the esophagus in a manner similar to that of the fibers of the anal sphincter. As they go posteriorly, they decussate and are attached to the vertebrae. The fibers of the pillars also form more or less the posterior layer of the lesser peritoneal cavity at the region of the vertebrae. In some instances, I have found on the posterior surface of the stomach a small triangle the sides of which are made up by the fibers of the pillars and the base by the posterior wall of the esophagus and the stomach. The size of the triangle varies in different subjects. In the dog it is usually small and at times absent; in man it is larger and measures from 1 to 2 cm. in length. The sides of the triangle are jointed together by means of fibrous tissue so that at times one may be able to admit an index finger through this potential opening.

In order to study the functional mechanism of these pillars and the parietal diaphragm, I made a gastrostomy and through that I inserted my finger into the esophagus. With the finger in this position, I noticed that coincident with each respiratory movement I could feel a

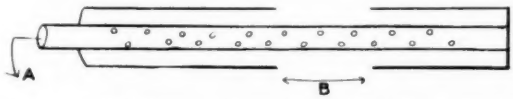

Fig. 4.—Diagram of tambour for recording diaphragmatic contractions. *A* indicates opening to recording tambour and *B*, the area covered with rubber finger.

progressively downward constriction at the site of the pillars. These contractions gave one the impression of milking contractions. With deeper respirations and when the animal strained, this milking effect became more evident. In order to determine whether or not this contraction was independent of the esophageal contractions, I paralyzed the diaphragm and noticed that these milking contractions stopped. By stimulating the vagus nerve peripherally or the esophagus I could get a contraction, but this time an entirely different type. This contraction was of the constricting type without any downward effect.

In order to determine whether or not the whole diaphragm was responsible for the milking effect or just the pillars, I stimulated the phrenic nerve at various levels and noticed a massive downward movement of the peripheral portion and, to a less extent, the central portion of the diaphragm. As a result of this contraction, the whole stomach and the liver were pushed down. When, on the other hand, the fibers of the pillars were stimulated directly from either the thoracic or the abdominal side, I noticed the characteristic milking contraction.

Under no circumstances could any contractions be induced if any portion of the cardiac end of the stomach was stimulated. By this

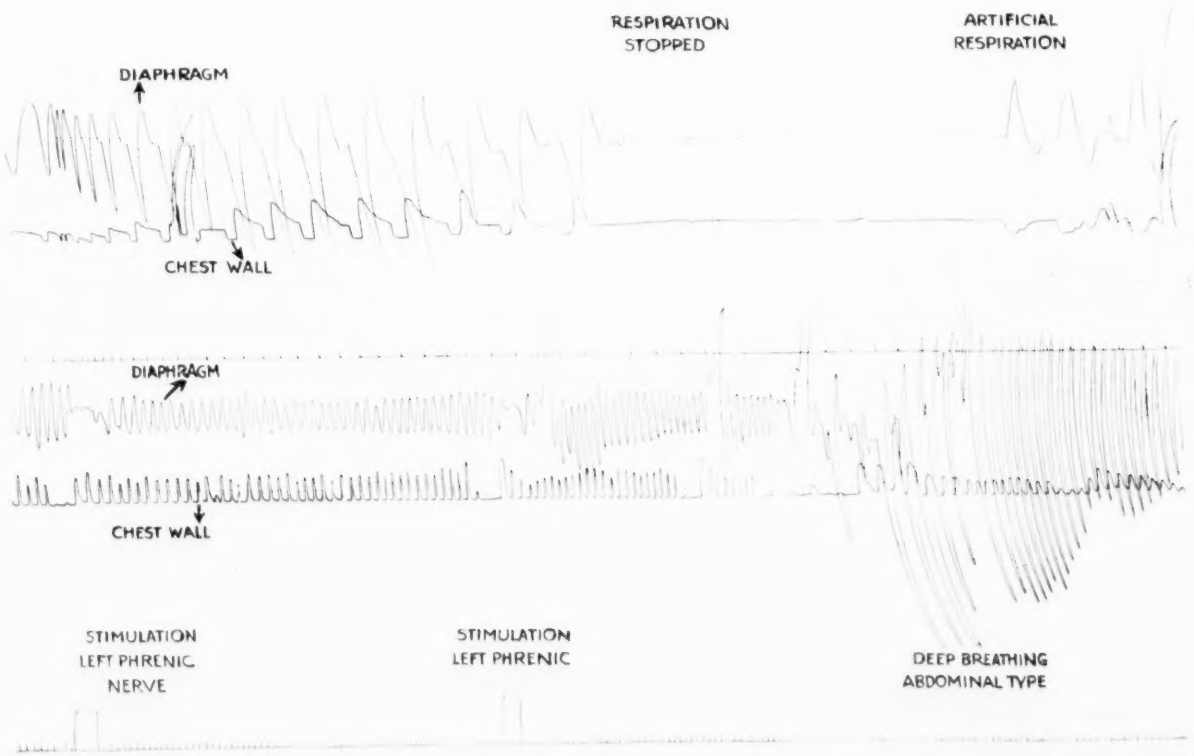

Fig. 5.—Lower record shows synchrony of respiratory wa ves of the chest wall and the diaphragm. Stimulation of the phrenic nerve shows a momentary cessation of the movements of both the chest wall and the diaphragm. Deep respirations of the abdominal type show the corresponding change in the amplit ude of the diaphragmatic waves. The upper record shows a synchronous cessation of the waves of the chest wall and diaphr agm when the respiration stopped.

means I could determine accurately the exact location of the esophageal-gastric junction. Stimulation of the esophagus up to the point of the junction would induce the characteristic contraction of the constricting type. Stimulation distal to the junction would not produce any effect.

In the next series, I tried to inflate the stomach by inducing a pharyngeal artificial respiration under otherwise normal conditions. I was unable to produce the acute distention that I encountered in cases in which operations on the lung had been performed. I also failed to produce distention even when I inserted an esophagoscope and pumped

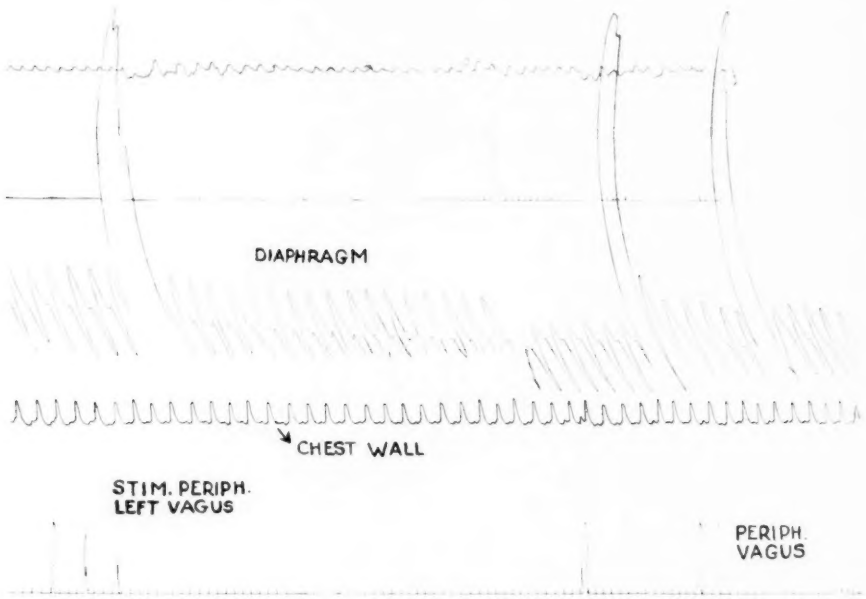

Fig. 6.—Stimulation of the peripheral end of the vagus nerve shows the effects of the constricting contraction of the esophagus without inducing any change on the waves of the chest wall.

compressed air into the stomach. If, however, the diaphragm, the lungs or the phrenic nerve was manipulated under conditions already mentioned, an acute dilatation of the stomach promptly developed. When the stomach is distended under these conditions, little air escapes from either the cardiac or the pyloric end, with the result that the stomach becomes tense and drumlike. On opening the duodenum just beyond the pylorus it was noticed that little if any air escaped from that end. At the cardiac end the air could escape with less difficulty, but not until an esophagoscope was inserted could free escape of the air be noted.

This last observation leads one to believe not only that the sphincter action of the pillars at this junction is of the milking type, but that because of the peculiar shape of the diaphragm there is a one way valve effect so that contents entering the stomach cannot easily be squeezed out.

In order to obtain exact and permanent records of these contractions, I devised an oblong tambour which can be inserted into the esophagus at the hiatus and thus record these contractions. This tambour (fig. 4) was constructed by inserting one brass tube into another and so arranging the rubber dam that the contractions would be transmitted to a second tambour and thence to the kymograph. By this means, I was able to notice to my own satisfaction that the contractions at the hiatus were homologous and synchronous with the excursions produced by a pneumograph that records movements during expansion of the chest. One could easily use this method for recording respiratory movements by making a gastrostomy and inserting the pneumograph into the gastro-esophageal junction (figs. 5 and 6).

SUMMARY

1. There is an intimate relationship between the pillars of the diaphragm at the hiatus esophageus and the gastro-esophageal junction.

2. The pillars exert a milking contraction on the gastro-esophageal junction and thus prevent the gastric contents from spilling backward into the esophagus.

3. The milking contraction is observed after stimulation of the pillars of the diaphragm or after stimulation of the trunk or the branches of the phrenic nerve in the immediate neighborhood of the pillars.

4. Stimulation of the vagus nerve or the esophagus produces a circular constriction at the gastro-esophageal junction. Stimulation of the vagus on the abdominal side or stimulation of the cardiac end of the stomach does not result in any contraction at the junction.

5. An acute dilatation of the stomach was encountered with the following conditions: (1) at the end or during a lobectomy or pneumectomy when there was an undue amount of rough handling of the lung; (2) as a result of unnecessary handling of the diaphragm, especially after downward retraction of the muscle; (3) in operations on the lung during which a resection of the phrenic nerve was made at the beginning of the operation, and (4) in a small number of cases of prolonged deep anesthesia when shock occurred or when there was a pharyngeal type of artificial respiration with positive pressure.

NORMAL RHYTHM OF WHITE BLOOD CELLS IN WOMEN*

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This study was made to determine whether or not the physiologic process of menstruation may influence the regular daily rhythm of the white blood cells, which has been shown to exist independently of other physiologic factors such as digestion and ordinary exercise (Sabin, Cunningham, Doan and Kindwall,[1] 1925, and Shaw,[2] 1927). The results obtained by these authors in regard to the effect of digestion were confirmed, and it was further found that menstruation does not appreciably affect the rhythm of the white blood cells, although there is a lymphocytic and leukocytic infiltration of the endometrium preceding the onset of menstruation (Novak and Te Linde,[3] 1924). This is taken as indicating that this rhythm is an essential characteristic of the white blood cells and is one phase of the fundamental rhythmicity of the vital activities of the body.

In the course of this investigation, it was found that a type of cell identified as a young eosinophil was present, which was basophilic in reaction and which was closely allied to the lymphocytic group of blood cells.

METHODS

Data regarding variations in the number of the white blood cells were obtained from two persons, five series of counts being made on each. Three of the series in the case of subject A and two in the case of subject B consisted of the first three days of menstruation. The third series of subject B included the day preceding the onset of menstruation and the first three days of menstruation. The fourth series in both cases included two consecutive days in an intermenstrual period, and the fifth was a series taken during menstruation when meals were omitted. The fifth series consisted of the first day of menstruation in the case of subject A, and of the first three days in the case of subject B.

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* From the Walter H. Merriam Laboratory, Department of Zoology, Mount Holyoke College.

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1. Sabin, F. R.; Cunningham, R. S.; Doan, C. A., and Kindwall, J. A.: Bull. Johns Hopkins Hosp. **37**:14, 1925.

2. Shaw, A. F. B.: J. Path. Bact. **30**:1, 1927.

3. Novak, E., and Te Linde, R. W.: Endometrium of Menstruating Uterus, J. A. M. A. **83**:900 (Sept. 20) 1924.

In one case (April 17, 1927, during menstruation), a count was made while the subject had an attack of gastro-enteritis. Food, except in the fifth series, and water in all series were taken as in ordinary daily life. Breakfast on some days was omitted; on others it was eaten between one-half hour and ten minutes before the first count was made. Lunch was eaten between four and six hours after the beginning of the count. On some days, food in small quantities was eaten occasionally. Both subjects took an active part in the counting and technical work involved in the experiments, and a moderate amount of exercise, as in ordinary daily life, was taken in all cases. In all, there are counts for twelve days on subject A and for fifteen days on subject B.

The methods used were essentially those described by Sabin, Cunningham, Doan, and Kindwall,[1] with minor modifications. Total and vital differential counts were made at fifteen or twenty minute intervals, for eight hour periods. Total counts were made with U. S. standardized pipets. The half-per cent acetic acid used for diluting the blood was made each morning before the counts were begun. Two of the workers alternated in making the total counts and the vital differential counts, but in all series the same person made total counts on one subject to eliminate the personal equation as far as possible. One of the persons made vital differential counts throughout. Fixed smears for differential counts were made at the same time that blood was taken for the total and the vital differential counts. From 200 to 250 cells were counted on the fixed smears. The vital preparations were made according to the technic described by Cunningham, Sabin, Sugiyama, and Kindwall[4] (1925), and 100 cells were counted on each slide.

TYPES OF WHITE BLOOD CELLS

In this study, the white blood cells which have been seen have been classified into four main groups: polymorphonuclear leukocytes, lymphocytes, monocytes and endothelial cells. The leukocytes have been further divided into neutrophils (both active and nonmotile), eosinophils and large and small basophils. The lymphocytes have been divided into small, intermediate, large and stimulated. The cells have been found to follow closely those described by Sabin[5] (1923), so that only those are discussed in this paper in which differences have been noted, or where it has been thought that more emphasis should be given to certain details.

Nonmotile Leukocytes.—In studies on both supravital and fixed preparations of blood, Sabin, Cunningham, Doan and Kindwall[1] have observed that nonmotile cells occur at times in large numbers. They have found such "showers" of nonmotiles both in heart's blood (rabbit) and in peripheral blood (human, finger; rabbit, vein of the ear). Their conclusion is that the neutrophils die in the circulation in great numbers, and that "these showers of nonmotile or dying cells represent a most important factor in the physiology of the blood."[6]

4. Cunningham, R. S.; Sabin, F. R.; Sugiyama, S., and Kindwall, J. A.: Bull. Johns Hopkins Hosp. **37**:231, 1925.

5. Sabin, F. R.: Bull. Johns Hopkins Hosp. **34**:277, 1923.

6. Sabin (footnote 1, p. 51).

In the present study, nonmotile cells have been seen in good smears but more often in poor ones. Occasionally a nonmotile is found on the same field with an active neutrophil or on the next field, and such cells have been considered neutrophils which have died in the circulation. However, marked showers were not observed. Nonmotile cells are rare on fields in which active cells are present and in which there is room for active movement. They have generally been found in thin places in an uneven smear or in the thin smears which result from using too small a drop of blood. These observations lead us to the conclusion that nonmotile cells result more often from technic and less often as neutrophils dying in the circulation.

Basophils and Eosinophils.—The significance of the basophil is a much disputed point. Numerous investigators regard it as a specific cell of equal rank with the other two types of granular leukocytes (Downey,[7] 1914; Ringoen,[8] 1915; Bunting,[9] 1922; Maximow,[10] 1924; Sabin, Cunningham, Doan and Kindwall [1]). On the other hand, many authors believe the basophil to be a degenerate or immature form of one of the other types of white cell. The question is complicated by the fact that eosinophilic granules may pass through a basophilic stage, and the question arises whether the basophils of the blood are these immature eosinophilic cells or whether they possess specific granules of different origin. Weidenreich [11] considered that they are degenerate lymphocytes in most animals. The negative peroxidase reaction of the basophilic granules correlated with progressive degenerative nuclear changes is interpreted by Graham [12] (1920) as indicating that the basophil is a degenerate eosinophil or, rarely, a neutrophil. Doan and Sabin [13] (1926), however, did not draw the same conclusion from this fact. Sometimes Graham found cells in which some of the granules reacted positively and the rest negatively; or in some cases the individual granules gave a mixed reaction, that is, the center reacted positively and the periphery negatively to the peroxidase test. This metachromatic reaction is of especial interest in view of a recent paper by Jordan [14] (1926).

From studies on fixed material of frog and teleost intestine, Jordan [14] classified the tissue basophils of the mucosa according to whether the granules stain blue (orthobasophilic) or purple (metachromatically basophilic) in Giemsa stain. The cells with orthobasophilic granules are

7. Downey, H.: Abstr. in Anat. Record **8**:135, 1914.
8. Ringoen, A. R.: Anat. Record **9**:233, 1915.
9. Bunting, C. H.: Physiol. Rev. **2**:505, 1922.
10. Maximow, A. A.: Physiol. Rev. **4**:533, 1924.
11. Weidenreich, F.: Anat. Record. **4**:317, 1910.
12. Graham, G. S.: J. Exper. Med. **31**:209, 1920.
13. Doan, C. A., and Sabin, F. R.: J. Exper. Med. **43**:839, 1926.
14. Jordan, H. E.: Anat. Record **33**:89, 1926.

thought to be immature eosinophils which develop from connective tissue cells. As they mature, the granules gradually become acidophilic and react somewhat to the eosin, presenting a metachromatically basophilic (purple) appearance. If at any time in this process of maturation degenerative or abortive changes occur, the granules also become metachromatic. When mature, the granules stain in eosin with no trace of blue. Although a contrary view is held by many authorities (Bunting [9]), Jordan does not believe the tissue basophil to be sharply distinct from that of the blood, and consequently his observations on the one are applied to the other. Jordan and Speidel [15] found that eosinophils develop by way of an intermediate basophilically granular stage from lymphocytes or connective tissue in amphibians and fish. Further evidence that eosinophils may be derived from hemoblasts is presented by Latta [16] (1921) in a study of the tonsil of the rabbit.

In the present study of human blood, two types of basophils have been found. The first is a large cell which is apparently the type referred to by Sabin [1] as an actively functioning cell, and by Graham [12] as a degenerate eosinophil or, rarely, a neutrophil. The granules of the large basophil are spherical and vary in size between those of neutrophils and eosinophils; they are acidic, since they stain a deep, slightly purplish red in vital neutral red, and bluish to reddish purple in Wright's stain. Basophils have not often been seen in motion. When they do move, it is with an ameboid motion, the granules going ahead of the nucleus.

The small basophil seen in the living state is quite different in appearance and behavior from the large basophil and probably belongs to a totally distinct group both in mode of origin and in later development. The study of this cell demonstrates strikingly the advantage of the supravital method of studying cells over the fixed-smear method, since in fixed smears it is entirely similar to the large basophil except in size. In numbers they have been found to vary from 0 to 4 per cent of the white cells, the average percentage being 0.6.

In vital preparations, it is a small actively motile cell which moves mainly by a streaming of the protoplasm, although sometimes clear lobate pseudopodia have been seen at the posterior end. In old or overheated preparations this cell may become round and motionless, as do other cells under the same conditions. When the cell is active, the nucleus moves ahead of the main body of cytoplasm; sometimes a few granules dart about in front of the nucleus. The nucleus is similar to the deeply indented nucleus sometimes seen in lymphocytes; at times it appears bilobed. The granules are spherical and vary in size from tiny specks

15. Jordan, H. E., and Speidel, C. C.: Am. J. Anat. **32**:155, 1923; **33**:485, 1924. Jordan (footnote 14).

16. Latta, J. S.: Am. J. Anat. **29**:159, 1921.

to bodies almost as large as the granules of an eosinophil. They do not react uniformly to neutral red, since in some cells they are deep red and not refractive, while in others they are lighter. More often the periphery takes the deep maroon color of basophilic granules, while the center is lighter. Such granules are often refractive when the focus is at the periphery or above the cell. They are believed to be similar to the metachromatic granules of Jordan's [17] developing eosinophil. Their appearance is much like that of Graham's [18] peripherally stained metachromatic granule, which however, he called degenerate instead of immature. Again, the granules sometimes resemble those of the eosinophil in color and refractivity. However, in spite of the differences in granules exhibited by individual cells in supravital preparations, these small cells surely belong to one group. Such cells do not contain mitochondria and are not phagocytic, since vacuoles are not present.

In fixed smears these cells appear as small basophils with metachromatic (purple) granules and but for their small size might be confused with ordinary large basophils. The evidence seems to indicate that these small cells are stages in the development of eosinophils from lymphocytes. Their close relationship to the lymphocytic group seems clear, since they move with the nucleus ahead, as do lymphocytes, and the nucleus itself resembles the lymphocytic nucleus. Such cells also may have clear hyaline pseudopodia. The lack of mitochondria is probably due to their disappearance before the maturing eosinophil reaches the peripheral circulation.

The question of the specificity of the types of white blood cell will not be discussed in this paper. A discussion of the leukocytes by Bunting,[9] together with recent articles by Maximow [10] and Jordan,[14] who support the monophyletic theory of blood cell origin, and by Cunningham, Sabin and Doan [19] (1925), who uphold the dualistic or polyphyletic theory, have appeared. The facts already presented indicate that eosinophils may differentiate from lymphocytes, although they, like the other granulocytes, have been considered myelocytic in origin. This apparent double origin of eosinophils is one argument against strict specificity of the white blood cell groups.

Lymphocytes.—Sabin's classification of lymphocytes into small, intermediate and large has been followed, except that in vital studies the large and intermediate forms with increased phagocytic activity have been classified separately as "stimulated lymphocytes" (Cunningham, Sabin and Doan,[19] Sabin [5] and Sabin, Cunningham, Doan and Kindwall [1]).

17. Jordan (footnote 14, p. 101).

18. Graham (footnote 12, fig. 7).

19. Cunningham, R. S.; Sabin, F. R., and Doan, C. A.: Contribution to Embryol. no. 84, Carnegie Inst., Washington **16**:229, 1925.

Stimulated lymphocytes differ mainly from the ordinary large and intermediate forms in having the cytoplasm crowded with neutral red vacuoles which are smaller than the vacuoles of neutrophils and monocytes. They are of two general types. The first kind closely resembles a monocyte in having a bean-shaped nucleus and neutral red vacuoles clustered in the indentation of the nucleus. It might be confused with a monocyte in which the roset arrangement of natural red granules and vacuoles around the centrosphere is obscured by the movement of the cell, or by an increased number of vacuoles, except that the mitochondria of lymphocytes are intermingled with the red-staining vacuoles, whereas in the monocyte the mitochondria are always peripheral to the roset of granules and vacuoles. The second type of stimulated lymphocyte has a lobed nucleus. If the vacuoles become numerous, the mitochondria may be obscured, and at first the beginner may confuse the cell with a basophil. Stimulated lymphocytes in fixed smears appear as ordinary large or intermediate lymphocytes. The stimulated lymphocyte is particularly mentioned here because it occurs more frequently than the literature indicates, and a knowledge of its structure is most important in the diagnosis of lymphocytes and monocytes. Maximow [10] found similar cells in conditions of inflammation.

Sabin [5] observed large lymphocytes dividing amitotically in the blood stream in a case of lymphoid leukemia. In this study, dividing lymphocytes have been seen occasionally.

Monocytes.—Monocytes are characterized by a bean-shaped nucleus. They have a peculiar roset of fine granules and larger vacuoles arranged radially about the centrosphere in the *hof* of the nucleus. Mitochondria are present and are always peripheral to the cluster of red-staining bodies. The latter detail of arrangement distinguishes the monocyte from the stimulated lymphocyte, but even this criterion is sometimes difficult to apply, because a monocyte may contain so large a number of vacuoles that the centrosphere is obscured; or the mitochondria may lie above the roset of red bodies near the surface of the cell, and it is only by careful focusing that they are seen not to be intermingled with them. Monocytes do not by any means always show their characteristic structures with diagrammatic clearness.

The monocyte moves by means of an undulating membrane, which, according to Carrel and Ebeling [20] (1926), is similar to that of sarcoma cells, macrophages and monocytes which have been cultivated in flasks. Although this membrane is seen best under the dark field microscope, it has been found that the outline as well as the filiform pseudopodia may be seen in part with direct illumination. Some movement may be detected, although the wavelike motion described by Carrel is not appar-

20. Carrel, A., and Ebeling, A. H.: J. Exper. Med. **44**:285, 1926.

ent. In fact, the undulating membrane has been an aid in diagnosis of cells which might be confused with stimulated lymphocytes.

VARIATIONS IN THE NUMBERS OF WHITE BLOOD CELLS

Observations.—Normal Rhythm and the Influence of Menstruation: Previous studies of the white blood cells have usually included serial counts on different persons or isolated ones on the same person. In this investigation, serial counts on consecutive days have been made on two persons. While no two curves are identical, certain features are constantly present. First, the number of white blood cells is markedly greater in the afternoon than in the morning. This increase begins in the later part of the morning. Only three exceptions (curve 3-2-27, chart 3; curve 3-25-27, chart 5; curve 5-17-27, chart 6) to this general rule occur in the twenty-seven curves included in this paper. In these three curves, the highest points come before 12 o'clock. In preliminary work, including eleven curves, an exception was not found. Second, there is an approximately hourly rise and fall in numbers of cells, which is independent of, and does not affect, the general tendency toward an afternoon rise. Third, the greatest number of cells at any time during the day is approximately twice the lowest number for the same day. These observations confirm the three points emphasized by Sabin, Cunningham, Doan and Kindwall [1] in regard to the rhythm of the total number of white cells. Fourth, there is often, though not invariably, a fall in the number of cells after the preliminary morning rise and before the height of the afternoon rise. This has also been noted by Sabin and her co-workers, in their charts 1A, 5E and 6F [21] and by Shaw.[22] There is no detailed correspondence between curves for counts on consecutive days. As already stated, no two curves are identical, and rhythms of consecutive days are not any more similar than rhythms of days a week or a month apart.

However, each person has a curve of characteristic pattern (compare charts 1 and 2). The counts of subject A are higher than the counts of subject B. The lower limit for subject A is from 5,050 to 9,350 cells, while the lower limit for subject B is between 4,025 and 6,132. The upper limit for subject A is from 9,475 to 13,425, and for subject B from 7,505, to 10,425, not including the high number of 11,550 which was reached during an attack of gastro-enteritis or the high count of 13,775 (curve 5-15-27, chart 6). The last curve is quite different from the other curves of subject B, showing much higher counts. The count was made on the first day of menstruation, and not any food was taken. The counts for the two days following were normal. Although the characteristic

21. Sabin et al. (footnote 1, pp. 39, 40).

22. Shaw (footnote 2, p. 7).

curves of subject A do not fluctuate more irregularly than do those of subject B, their wider range permits of greater fluctuations, which result in a curve pattern different from that of subject B. It is interesting to note that the individual patterns are constantly exhibited; for example, in curve 3-29-27 (chart 4) the pattern is characteristic for subject A, although the zone of fluctuation is between 9,350 and 13,425 cells. These extremely high numbers did not result from any apparent

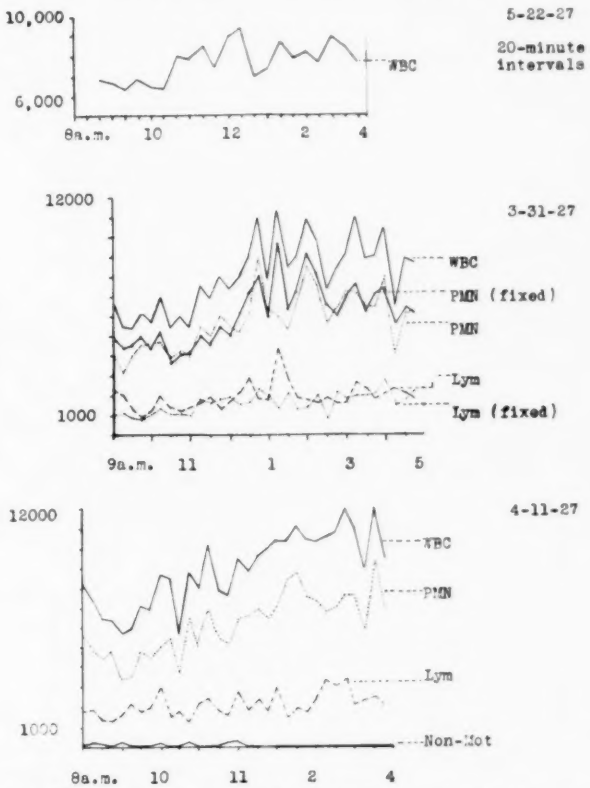

Chart 1.—Exact curves of total and differential counts of subject A. Curve 4-11-27 represents a day's count during an intermenstrual period and curves 5-22-27 and 3-31-27 are of counts taken during menstruation. Curve 5-22-27 is for a day when meals were omitted.

The total and differential counts for each day have been plotted graphically (charts 1 to 4) and the curves numbered with the date on which the count was made. The times of day are given on the abscissas and the numbers of cells on the ordinates. *L* indicates lunch and *B*, breakfast, and the figures beneath the date of the curve indicate the time when breakfast was taken. The vertical lines drawn through the curves show when food in small quantities was eaten (candy, crackers, etc.). The vital stain was used unless otherwise stated.

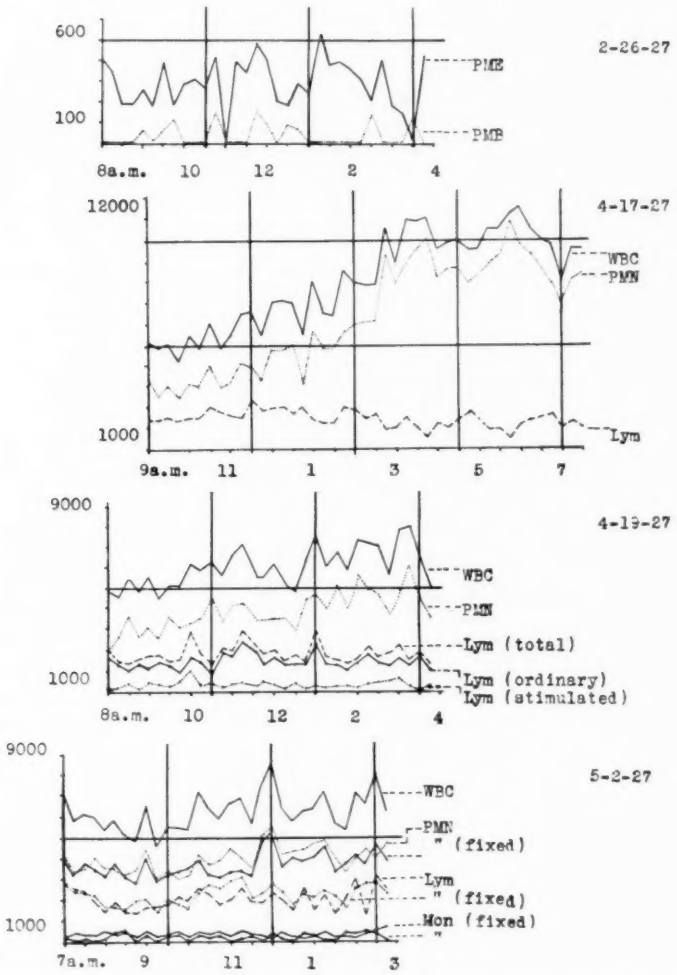

Chart 2.—The exact curves of total and differential counts of subject B. Curve 5-2-27 represents a day's count during an intermenstrual period, and curves 2-26-27, 4-17-27 and 4-19-27 are counts taken during menstruation. Curve 4-17-27 is peculiar in that the subject had an attack of gastro-enteritis while the counts were being made.

cause. Counts on both subjects, under somewhat different conditions six months later, exhibited the same individual patterns. Another consequence of this wider range in the counts of subject A is that the afternoon rise is much more pronounced than in the case of subject B. Individual differences in numbers of blood cells were noted by Rous (1908)[23] in a discussion of the quantities of lymphocytes supplied to the blood of the dog. He said that "the tissues producing the lymphocytes are "set" at a rate of activity definite in the individual."[24] Shaw[2] found that in human blood "the total number of leukocytes is influenced by the personal factor."[25] On the whole, the curves of subject A are slightly more irregular during menstruation (curves 3-2-27, 3-3-27, 3-4-27, 3-29-27, 3-30-27, 3-31-27, 4-25-27, 4-26-27, 4-27-27, charts 3 and

High and Low Counts of White Blood Cells

Subject A			Subject B		
Counts		Range of Count	Counts		Range of Count
Normal	4-10-27	5075-10775	Normal	5- 1-27	4800- 9382
Normal	4-11-27	5700-12075	Normal	5- 2-27	4500- 8670
Menstruation	3- 2-27	6933-10917	Menstruation	2-24-27	5266- 8800
Menstruation	3- 3-27	6390-12500	Menstruation	2-25-27	5600- 8932
Menstruation	3- 4-27	5375-11657	Menstruation	2-26-27	6132- 9666
Menstruation	3-29-27	9350-13425	Menstruation	3-23-27	5475-10425
Menstruation	3-30-27	6350-11044	Menstruation	3-24-27	5500- 8575
Menstruation	3-31-27	5424-11200	Menstruation	3-25-27	4625-10275
Menstruation	4-25-27	5050-12950	Day before menstruation	4-16-27	5390- 7505
Menstruation	4-26-27	5450-11200	Food poisoning	4-17-27	4200-11550
Menstruation	4-27-27	5175-11400	Menstruation		
Menstruation No food	5-22-27	6350- 9475	Menstruation	4-18-27	5125- 8825
			Menstruation	4-19-27	4525- 9125
			Menstruation No food	5-15-27	5150-13775
			Menstruation No food	5-16-27	5075- 8875
			Menstruation No food	5-17-27	4025- 8500

4) than are the normal curves (4-10-27 and 4-11-27, chart 3) as described in another paragraph. Such an effect is not apparent in the curves of subject B (charts 5 and 6).

Menstruation does not have a decided influence on the numbers or rhythm of the white blood cells. As already noted, the counts for this part of the work were made during the first three days of menstruation. The number of the cells is perhaps slightly increased, as seen in the accompanying table. The lower level of the menstrual counts is higher than the lower level of the normal counts, with the exception of 4-25-27 and 5-17-27. Three times in the case of subject A (3-3-27, 3-29-27, 4-25-27) and four times in the case of subject B (2-26-27, 3-23-27, 3-25-27, 5-15-27) the highest point of the menstrual counts is above the

23. Rous, F. P.: J. Exper. Med. **10**:238, 1908.

24. Rous (footnote 23, p. 268).

25. Shaw (footnote 2, p. 19).

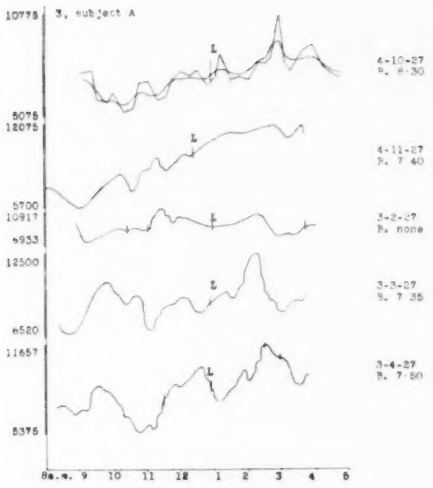

Chart 3

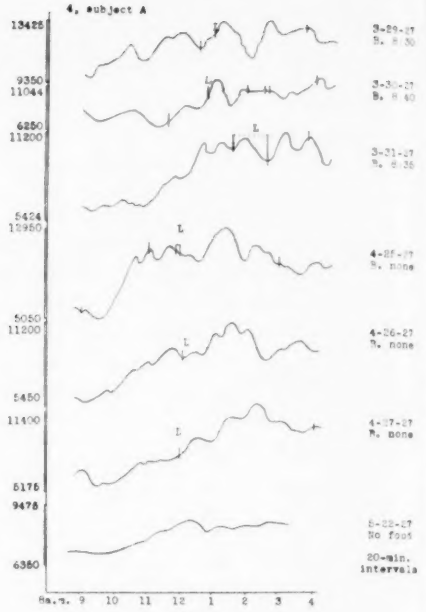

Chart 4

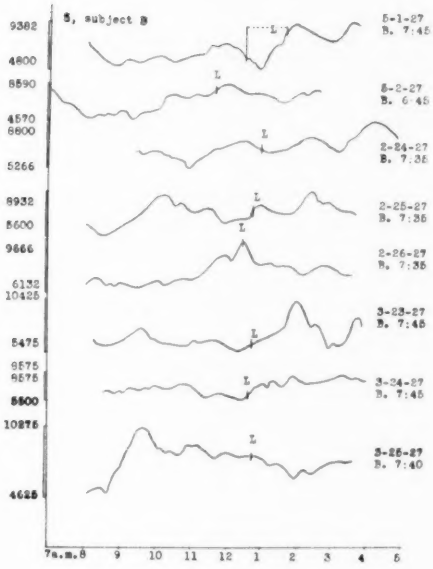

Chart 5

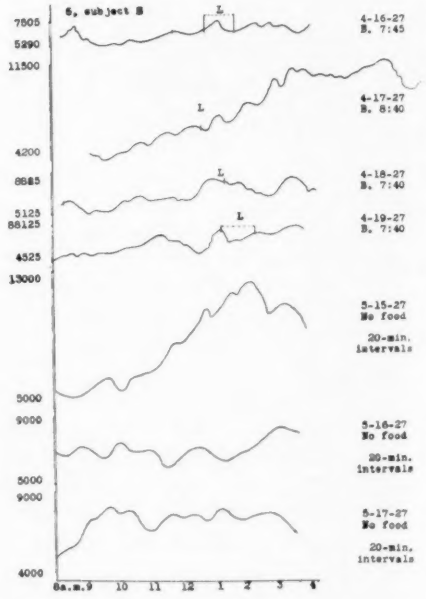

Chart 6

Charts 3, 4, 5 and 6.—These charts show general curves of total white counts which are representations of the exact curves. Curve 4-10-27 shows an exact curve superimposed on its flattened curve. These flattened curves are used to show the larger fluctuations and general tendencies.

highest point of the normal counts. However, this difference is so slight and inconstant that a definite conclusion cannot be made.

The menstrual curves of subject A present larger fluctuations than the normal curves. The normal curves (4-10-27, 4-11-27, chart 3; 4-11-27, chart 1) are more even; the afternoon rise follows the mean level fairly regularly and is not indented by deep depressions, except from 3 to 4 o'clock. On the contrary, the afternoon counts of the menstrual curves (3-2-27, 3-3-27, 3-4-27, chart 3) alternate fairly rapidly between high points and low points, the latter being almost as low as the low points of the morning counts. The morning counts of normal and menstrual curves are similar.

Afternoon Leukocytosis: Digestion apparently is not the cause of the afternoon leukocytosis. A study of the curves shows that breakfast does not influence the white blood cell picture, since a constant difference is not noticed between the curves for the days when breakfast was eaten and those for the days when breakfast was omitted. That lunch likewise is not a constant factor is seen by the fact that the highest point of the afternoon rise may come at any time and does not bear any relation to the time of eating lunch (charts 2, 4, 5 and 6). The small quantities of food eaten irregularly do not have a marked effect, since on some days no food except breakfast and lunch was eaten; yet the respective curves do not present constant variations due to this factor.

That the afternoon rise is not due to digestion is conclusively shown by a day's count made on subject A (curve 5-22-27, charts 1 and 4), when she ate nothing from dinner the night before (except an orange at midnight) till the end of the count, and by two days' counts made on subject B, when she ate nothing between dinner the night before and the end of the count. A third day's count made under similar conditions on subject B showed a morning rise (curve 5-17-27, previously referred to). Curve 5-22-27 (subject A, chart 1) is representative of these counts, with due allowance for the lower numbers of cells in the case of subject B. It shows quite as marked an afternoon rise as do the curves of days when food was not eaten.

A condition of fatigue apparently does not have a constant influence on the white blood cell count. From five to six hours was the amount of sleep taken the nights before the counts shown in curves 3-23-27, 3-24-27, 3-25-27 (chart 5) and 4-18-27 (chart 6). While curve 3-25-27 is the reverse of the typical curve, in having its high points in the morning, the other counts made under similar conditions do not show this characteristic; again, the counts shown on another reverse curve (3-2-27, chart 3) were made on a day following the normal amount of sleep (between seven and eight hours).

Physiologic response to a digestive disturbance has a marked effect on the numbers of the white blood cells, although the hourly rhythm

is maintained; this is in contrast to the slight or negative influence of normal physiologic processes (menstruation, digestion). Curve 4-17-27 (charts 2 and 6) shows the increase in numbers due to an attack of gastro-enteritis, the symptoms of which lasted from 12 to 4 o'clock.

Observations on the Differential Counts: 1. Neutrophils. The observations of Sabin, Cunningham, Doan and Kindwall [1] and of Shaw [2] that the curve of the neutrophils follows that of the total white cells is confirmed in this study.

The observations already recorded concerning the influence of physiologic factors (menstruation, digestion and fatigue) on the total number of white cells hold true specifically for the neutrophilic rhythm; that is, (1) menstruation possibly causes a slight increase in numbers in both cases, and greater irregularity in rhythm in the case of subject A; (2) digestion does not have a constant effect on the numbers or rhythm of the cells, and (3) fatigue does not have a constant effect on the numbers or rhythm of the cells.

In contrast to the observations of Sabin, Cunningham, Doan and Kindwall [1] the nonmotile neutrophils, as already discussed under "Types of White Blood Cells," were not found in sufficiently large numbers to influence the neutrophilic, and therefore indirectly, the total white curve (curve 4-11-27, chart 1).

2. Basophils and Eosinophils. These cells occur in small numbers (basophils, from 0 to 2 per cent and eosinophils, from 0 to 5 per cent) and are frequently absent in counts of a hundred cells. There seems to be no regularity in the variations of their numbers (curve 2-26-27, chart 2).

3. Lymphocytes. As noted by Sabin, Cunningham, Doan and Kindwall,[1] the numbers of the lymphocytes oscillate in a half hourly rhythm, but there is no constant rise in the mean level correlated with the time of day (curves 3-31-27, 4-11-27, chart 1; 4-17-27, 4-19-27, 5-2-27, chart 2). Menstruation, digestion and fatigue do not have any effect on their numbers or their rhythm. The stimulated and ordinary types of lymphocytes are plotted separately on curve 4-19-27 (chart 2). From this it is seen that the curve for the ordinary lymphocytes closely follows the total curve, much as the neutrophilic curve follows the total white curve. The stimulated lymphocytes, being fewer in number, do not influence the total lymphocyte curve so greatly. The ratio of stimulated to ordinary lymphocytes is practically the same on all curves and therefore is not influenced by different physiologic conditions. There was also no correlation between the numbers of stimulated lymphocytes and the time which elapsed between the making of the slide and the actual count. Therefore, they are not expressions of degenerative changes, but rather seem, as Maximow [10] said, to represent a greater degree of functional

activity of the cell. Curves 3-31-27 (chart 1) and 5-2-27 (chart 2) show the correlation of fixed and vital lymphocyte counts.

4. Monocytes. The observations of Sabin, Cunningham, Doan and Kindwall [1] that the monocytes tend to have an hourly rhythm similar to the rhythm of the neutrophils have been confirmed. Variations do not occur in connection with the time of day or with different physiologic factors. Curve 5-2-27 (chart 2) shows the correlation of fixed and vital monocyte counts.

Comment and Review of Literature.—As Sabin, Cunningham, Doan and Kindwall [1] have pointed out, much of the previous work on the determination of the normal number of blood cells has been to find the average number rather than the normal range in numbers for an individual. Doan and Zerfas [26] (1927) emphasized the necessity of the "zonal concept" in blood counts and, according to the experiments recorded in this paper, this point of view is valid.

From the data obtained in this work, we believe that there is not only a zone of variation for each person but a characteristic pattern as well. Although no two curves are identical and although curves for consecutive days are no more alike than curves for days a week or a month apart, curves for each person possess a similarity which can be seen readily. The wider range or zone of one individual results in a curve with marked acute depressions and peaks, whereas the narrower range of the other makes for a more even curve. This pattern concept is upheld by comparison of counts made six months apart on the same two subjects. The striking conformity of the later curves to the pattern of those given in this paper is more than a coincidence.

These studies also confirm the observations of those workers who believe that there is a characteristic rhythm of the white blood cells, both total and differential, and that the increase in the afternoon is not dependent on food intake. Although these experiments were not primarily undertaken to investigate digestive leukocytosis, some points may be noted which have a bearing on the subject. The numbers, rhythm and afternoon rise of the various types do not differ fundamentally on the days when food was not eaten, on the days when only breakfast and lunch were eaten, and on the days when small amounts were eaten irregularly in addition to breakfast and lunch. There is also no definite correlation between the mealtimes and the afternoon leukocytosis.

While it cannot be said absolutely that digestion may not have some effect on the numbers of the white blood cells, the conclusion is obvious that it does not have such a profound effect as has been thought. Whatever slight effects it may have in these counts cannot be determined since too many other factors are involved. The rhythmic variations in

26. Doan, C. A., and Zerfas, L. G.: J. Exper. Med. **46**:511, 1926.

the numbers of the white blood cells must then have some other underlying cause than digestion. Japha [27] (1900) was one of the first to record variations in the blood count regardless of digestion. Sabin, Cunningham, Doan and Kindwall [1] and Shaw [2] found that variations in the white count occur whether food is eaten or not. Sabin, Cunningham, Doan and Kindwall further found that these changes occur in an almost hourly rhythm during the day, with a total increase in the afternoon, and that the numbers of the different kinds of cells also vary in a typical rhythm. However, the majority of authors still believe that digestion causes a rise in the number of the white blood cells, as seen in the reviews of the literature given by Mitchell [28] (1915), Sabin, Cunningham, Doan and Kindwall,[1] Plichet [29] (1927) and Shaw.[2]

Another important physiologic factor to be taken into consideration when making experiments on women is menstruation. As has been previously noted, Novak and Te Linde [3] described a lymphocytic and a leukocytic infiltration of the endometrium just preceding the onset of menstruation. A statement is not made about the presence of white cells in the uterine wall on the first day of the menstrual period, but on the second the infiltration is said to be less than that of the early stages. At this time, lymphocytes are the predominant cells. Although such an infiltration may occur, it does not seem to be of sufficient magnitude to disturb the number of cells (unless it causes a slight increase) or their percentage in the peripheral blood. The curves for counts made during menstruation differ from each other as much as they do from curves for the days during intermenstrual periods.

It is current opinion that the greatest changes in the metabolism of women occur within the few hours preceding or immediately after the beginning of menstruation. Counts were made during seven cycles, but only two were made within the first hours after the onset. Curve 3-29-27 (chart 4) for subject A is of a count which was started one and one-half hours after the beginning of the period, but there is no difference in the form of the curve. The curve for subject B does not offer evidence in regard to this point, as it is the one which also shows the results of gastro-enteritis. All of the other counts were made several hours after the start of the menstrual flow. In their work on the studies of metabolism of women, Okey and her associates (1925, 1926 and 1927) [30]

27. Japha, A.: Jahrb. f. Kinderh. **52**:242, 1900.

28. Mitchell, A. G.: Leukocyte Counts During Digestion of Bottle-Fed Infants, Am. J. Dis. Child. **9**:358 (May) 1915.

29. Plichet, A.: Le sang. biol. et path. **1**:134, 1927.

30. Okey, R., and Robb, E. I.: J. Biol. Chem. **65**:165, 1925. Okey, R., and Erikson, S. E.: Ibid. **68**:687, 1926. Okey, R., and Boyden, R. E.: Ibid. **72**:261, 1927.

found the same difficulty. They were able to make determinations during the first few hours of menstrual bleeding in only a small percentage of cases.

Although in subject A the curves are slightly more irregular during menstruation, such an effect is not seen in the curves of subject B. Okey and Robb,[31] in their work on the blood sugar level during fasting noticed a greater deviation from the average during the menstrual period, although they did not find a consistent cyclic variation. Greisheimer [32] (1927) observed that the blood sedimentation rate of women is faster than in men but that it is not faster during periods than between periods.

It has been shown that there is some evidence for cyclic variations in the metabolism of women by studies on measured activities and on the chemistry of the blood. Moore and Barker [33] (1923) and Moore and Cooper [34] (1923) demonstrated low menstrual and high intermenstrual variations in muscular efficiency, in cardiovascular changes and in respiratory rate. Okey and Erikson [35] and Okey and Boyden [36] observed low menstrual values for blood uric acid and cholesterol. Total non-protein nitrogen was found to be high during or just after the period. The fatty acid tended to vary, but the determinations of lecithin were more nearly constant. Although from this brief summary it is seen that some of the constituents of the blood may exhibit certain cyclic changes in relation to menstruation, neither they nor the leukocytic infiltration of the uterine wall at that time produces any marked modification in the white blood cell picture.

Variations in the numbers of white blood cells have been ascribed to various physiologic factors. In this study, the slight or negative influence of digestion, menstruation and moderate fatigue bring out the fact that the rhythm of the white blood cells tends to be constant in different persons and to conform to characteristic patterns. It is thought that this rhythm is really a functional activity of the cells themselves, and cannot be explained as being caused, although it may be influenced, by extrinsic physiologic factors, just as the heart beat is not caused but may be modified by external factors.

Regarding the rhythms of the different kinds of white blood cells, it is apparent from the curves (charts 1 and 2) that the rhythm of the whole number of the white cells is determined by the rhythm of the neutrophils; this is to be expected, since they form the greater proportion of the white cells. The question, then, concerning the cause of the gen-

31. Okey and Robb (footnote 30, first reference).
32. Greisheimer, E. M.: Am. J. M. Sc. **174**:338, 1927.
33. Moore, L. M., and Barker, J. L.: Am. J. Physiol. **64**:405, 1923.
34. Moore, L. M., and Cooper, C. R.: Am. J. Physiol. **64**:416, 1923.
35. Okey and Erikson (footnote 30, second reference).
36. Okey and Boyden (footnote 30, third reference).

eral rhythm of the white cells narrows itself down to the cause of the rhythm of the neutrophils. This study does not confirm the belief of Sabin, Cunningham, Doan and Kindwall [1] that the neutrophils die out in the circulation in fairly large numbers, since the observations indicate that the dead nonmotile cell, although sometimes appearing as a dying neutrophil in the blood stream, is more often an artefact due to technic in the supravital smears. Neither is there any explanation for the half hourly rhythm of the lymphocytes, the hourly rhythm of the monocytes, the irregular rhythm of the basophils and eosinophils or the constant mean numbers of all four groups. It can only be said that the numbers of white blood cells in the peripheral circulation are maintained within certain limits and fluctuate regularly within these limits. This occurrence may be an expression of the fundamental rhythmicity of vital activities which Rogers [37] (1927) defined as "the tendency of the fundamental or even incidental operations of the cells of the animal body or of the organism to alternate or oscillate from one side to the other of certain mean values."

CONCLUSIONS

1. Evidence indicates that the small basophil may be an intermediate stage in the development of certain eosinophils of lymphocytic origin.

2. The characteristics of the normal rhythm of the white blood cells previously described have been confirmed in general, except that it is not believed that cells die in the peripheral circulation to such an extent that the neutrophilic curve is influenced by them.

3. The number of white blood cells varies within certain limits. These limits are different in different persons; under normal conditions, they are constant in a given person.

4. The curves representing the daily rhythm of the white blood cells have a characteristic pattern for each person.

5. The number and rhythm are essential characteristics of the white blood cell picture and are not caused or fundamentally modified by normal physiologic processes (digestion, menstruation) or different physiologic states of the normal body (mild fatigue, moderate exercise).

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37. Rogers, C. G.: Text-Book of Comparative Physiology, New York, McGraw and Hill, 1927, p. 69.

FATAL PHENOBARBITAL POISONING

REPORT OF A CASE WITH TOXICOLOGIC ANALYSIS *

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Although it is now fifteen years since its introduction into therapeutics, there does not appear to have been a case of fatal poisoning definitely attributable to phenobarbital so far recorded in the literature, despite the fact that it is undoubtedly the most widely used sedative and anticonvulsant drug.

A number of reports of toxic reactions and skin eruptions following the use of phenobarbital have appeared, but only one of these cases proved fatal, and this does not seem to have been attributable to the drug.

In 1926, Hamilton, Geiger and Roth [1] reviewed the literature on phenobarbital poisoning, and found only six cases reported previous to their own: two by Haug,[2] two by Farnell [3] and one each by Stein [4] and Hueber.[5] All of the patients recovered except that of Hueber, whose death was probably due to a flaring up of an old tuberculous condition. Toxic reactions other than those cited by Hamilton et al., however, were reported by Ungar,[6] Nicolai,[7] Weig [8] and Carlill.[9]

Still more recently, Jackson [10] reported six cases of acute phenobarbital poisoning, in all of which recovery occurred. There are also reports by Stone [11] of a case of phenobarbital poisoning simulating mul-

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*From the Department of Pharmacology, University of Minnesota.

1. Hamilton, E. S.; Geiger, C. W., and Roth, J. H.: Luminal Poisoning with Conjunctival Residue, Illinois M. J. **49**:344 (April) 1926.
2. Haug, W.: Zwei Fälle von Luminalvergiftung, München. med. Wchnschr. **66**:1494, 1919.
3. Farnell, F. J.: Luminal; Its Toxic Effects, J. A. M. A. **61**:192 (July 19) 1913.
4. Stein, J.: Case of Luminal Poisoning, Therap. Halbmonatsh. **34**:387 (July 15) 1920.
5. Hueber, D.: Ein Fall von Luminalvergiftung mit tödlichem Ausgang, München. med. Wchnschr. **66**:1090, 1919.
6. Ungar, T.: Ein Fall von Luminalvergiftung, Wien. klin. Wchnsch. **27**: 847, 1914.
7. Nicolai, H. W.: Phenobarbital Poisoning, Klin. Wchnschr. **2**:1891 (Oct. 8) 1923.
8. Weig, F. L.: Zur Luminalvergiftung, Deutsche med. Wchnschr. **51**:272, 1925.
9. Carlill, H.: Luminal Poisoning, Lancet **2**:596 (Sept. 19) 1925.
10. Jackson, A. S.: Toxic Reaction from Phenobarbital, J. A. M. A. **88**:642 (Feb. 26) 1927.
11. Stone, C. W.: Barbital Poisoning Simulating Multiple Sclerosis, Ohio State M. J. **23**:132 (Feb.) 1927.

tiple sclerosis and of two toxic reactions by Bollinger.[12] Menninger[13] reported three cases of skin eruptions which occurred among 400 patients.

The distribution of phenobarbital in the tissues does not seem to have been studied, but would presumably follow closely the distribution of the other barbituric acid derivatives. Fabre and Fredet[14] studied the distribution of barbital and allyl-isopropyl barbituric acid in dogs killed shortly after the ingestion of a hypnotic dose of the barbituric acid derivatives. These observers found the drug to be largely concentrated in the brain, with smaller quantities in the spleen, kidney and liver.

The question of the excretion of the barbituric acid derivatives in the urine has not at present been satisfactorily settled. In fourteen patients receiving barbital in amounts of from 0.5 to 2 Gm., Reiche[15] recovered from 0.18 to 11.7 per cent of the amount ingested from the urine in thirty-eight hours.

Amount of Phenobarbital and Allyl-Isopropyl Barbituric Acid Recovered from One Hundred Grams of the Organ

	Barbital	Allyl-isoprophyl Barbituric Acid
Brain	138 mg.	68 mg.
Spleen	44 mg.	32 mg.
Kidney	5 mg.	5 mg.
Liver	4 mg.	6 mg.

Luce and Feigl[16] and, later, Fleury and Guinnebault[17] determined the elimination of phenobarbital in dogs under continuous administration of from 0.2 to 0.3 Gm. daily but observed only the excretion of small quantities. Halberkann and Reiche[18] reported that after they gave 1.8 Gm. of phenobarbital to a series of patients, they recovered from 11 to 25 per cent of the ingested drug over a period of eight days.

12. Bollinger, H. J.: Toxic Reactions from Phenobarbital; Two Cases, California & West. Med. **26**:659 (May) 1927.

13. Menninger, W. C.: Skin Eruptions with Phenobarbital, J. A. M. A. **91**: 14 (July 7) 1928.

14. Fabre, R., and Fredet, P.: Studies in the Localization and Elimination of Some Derivatives of Malonyl Urea, J. de pharm. et de chim. **2**:321, 1923.

15. Reiche, F.: Zur Kenntnis der Veronalvergiftung und der Veronalausscheidung, Klin. Wchnschr. **5**:2112 (Nov. 5) 1926.

16. Luce, H., and Feigl, J.: Ueber Luminal-Exantheme, Therap. Monatschr. **32**:236, 1918.

17. Fleury, P., and Guinnebault, E.: L'elimination de la phenylethylmalonyluree, Comp rend. Soc. de biol. **93**:1508, 1925.

18. Halberkann, J., and Reiche, F.: Ueber die Ausscheidung einiger viel verwendeter Barbitalsäureverbindungen mit dem Urin, München. med. Wchnschr. **74**:1450, 1927.

Reinert [19] obtained about 20 per cent excretion over a period of ten days in dogs fed 1.5 Gm. of phenobarbital. The greatest excretion occurred during the third and fourth days. Wetselaar [20] reported that phenobarbital is not found in the urine in cases of acute poisoning.

REPORT OF CASE

L. C., a woman, aged about 35, was found dead in a room of a Minneapolis hotel. She had apparently been dead about twenty hours when found. Evidence of poison was not found in the room, but it was known that the woman had been in the habit of taking phenobarbital. The autopsy did not show anything significant. The stomach and its contents and portions of the liver, brain and about 50 cc. of urine were removed for toxicologic analysis. The urine was extracted several times with 25 cc. portions of ether, and the combined ether extracts evaporated to about 20 cc. This was then extracted with 5 cc. of five hundredth-normal sodium hydroxide. A small portion of this alkaline extract, after acidification with acetic acid, gave a faint turbidity with lead acetate solution, which would indicate the probable presence of a trace of one of the barbituric acid derivatives, but there was not a sufficient amount present for identification. It would appear, then, that in the case of phenobarbital, the excretion of the drug in the urine is too slow to serve either as a diagnostic aid in cases showing toxic reactions, as is possible with barbital poisoning (Leake and Ware [21]), or as a source of identification of the drug in the toxicologic analysis of fatal poisoning.

The stomach and liver were hashed separately, and extracted repeatedly with sufficient ether to cover the material. The combined ether extracts were filtered and evaporated to a volume of 40 cc. and extracted twice with 10 cc. of five hundredth-normal sodium hydroxide. Considerable care has to be exercised in making this extraction with alkali, as the mixture shows a marked tendency to emulsify, and if shaken violently, the whole mass becomes almost solid and the emulsion can be broken only by complete evaporation of both solvents.

The aqueous extract thus obtained still contained considerable impurities, chiefly split proteins and soaps which are soluble in alkaline solution.

The process of purification was continued, therefore, by acidifying the aqueous extract with 50 per cent acetic acid and extracting the slightly acid liquid several times with ether, most of the proteins and soaps being left in the water layer. The ether extracts, after filtration and concentration, were extracted with dilute alkali. As judged by the reaction of a few drops of this solution, after acidification with acetic acid to a solution of lead acetate, which precipitates both the barbituric acid derivative and the dissolved proteins, the amount of impurity present was now slight.

The alkaline solution was now made slightly acid with acetic acid. In the case of the extract from the liver, there was just sufficient phenobarbital present to exceed the solubility of this drug in water (1 mg. per cubic centimeter) and to give a faint turbidity to the solution, while the extract obtained from the stomach was not sufficiently concentrated to do this.

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19. Reinert, M.: Ueber die Ausscheidung von Barbitursäurederivaten im Harn beim Hund, Arch. f. exper. Path. u. Pharmacol. **130**:49, 1928.

20. Wetselaar, D. Y. K.: Pharm. Weekblad. **59**:521, 1922.

21. Leake, W. H., and Ware, E. R.: Barbital Poisoning, J. A. M. A. **84**:434 (Feb. 7) 1025.

In order to recover as much phenobarbital as possible, as crystallization from water did not appear feasible, the acid aqueous extracts were extracted several times with ether and the ether allowed to evaporate spontaneously. The residue obtained was a white powder, having a melting point of 170 C. Phenobarbital melts between 172 and 174 C.[22] The identity of the material obtained by extraction was established as phenobarbital by taking a mixed melting point of a 50-50 mixture of the residue and a known sample of phenobarbital (melting point, 172 C.). The melting point so obtained was 170.5 C. If the two substances had not been identical chemically, the melting point of the mixture would have been considerably depressed.

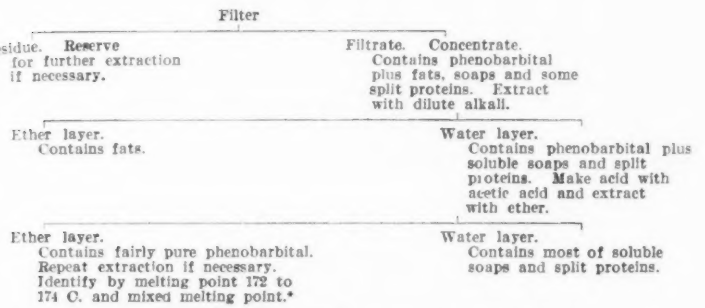
The amount of phenobarbital obtained from the stomach was from 5 to 6 mg. and from the liver from 15 to 20 mg.

Extraction of the phenobarbital from the brain presented somewhat more difficulty on account of the presence of large quantities of lipoids and fats, although the phenobarbital content of this tissue should be considerably higher. Portions of the brain were hashed and extracted for several days with ether. The ether extract was then removed and filtered, and the ether allowed to evaporate. The residue was taken up in 2 cc. of ether and poured into 50 cc. of alcohol and filtered. This removes most of the fats and the cephalin. The alcohol was then removed by evaporation on the water bath (or by distillation) and the residue taken up in acetone, in which the lecithin is insoluble. After filtration, the acetone was removed by evaporation and the residue taken up in a small quantity of boiling water and filtered. After the mixture was cooled in the icebox, a white precipitate was obtained, which, after recrystallization, melted at 170 C., and the melting point was not depressed by mixing with known phenobarbital.

The approximate amounts of phenobarbital isolated from the various organs per hundred grams was: stomach, from 3 to 4 Gm.; liver, 5 Gm. and brain, 90 mg.

The following schema would appear to be a satisfactory method for the toxicologic analysis of poisoning by phenobarbital:

Hash tissue and extract from three to five times with ether.


Filter
Residue. Reserve for further extraction if necessary.
Filtrate. Concentrate. Contains phenobarbital plus fats, soaps and some split proteins. Extract with dilute alkali.
Ether layer. Contains fats.
Water layer. Contains phenobarbital plus soluble soaps and split proteins. Make acid with acetic acid and extract with ether.
Ether layer. Contains fairly pure phenobarbital. Repeat extraction if necessary. Identify by melting point 172 to 174 C. and mixed melting point.*
Water layer. Contains most of soluble soaps and split proteins.

* If any difficulty is experienced in removing soaps, which may be carried over into the ether layer on extraction in acid solution, they may be readily removed by treating the acid aqueous solution with a slight excess of barium chloride solution and filtering off the precipitated barium soaps.

22. U. S. Pharmacopoeia, ed. 10, Philadelphia, J. B. Lippincott Company, 1926, p. 282.

SUMMARY

1. A case of fatal poisoning from phenobarbital is reported.

2. A method is presented for the toxicologic analysis of phenobarbital, including extraction and identification from the stomach, liver and brain.

3. Phenobarbital does not appear to be excreted in the urine in cases of acute poisoning.

MUSTARD GAS AND TUBERCULOSIS

AN EXPERIMENTAL STUDY *

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Since the introduction of toxic gases into modern warfare on April 22, 1915, all sorts of dire late sequelae have been declared the result of exposure to the various war gases. Peribronchial thickenings and scarring of the parenchyma of the lung have been much discussed; various functional disturbances have been attributed to the late effect of gassing. As a result of several years of experimental work, I [1] published data in 1925 which showed that there was little or no pathologic basis for the many symptoms attributed by the ex-soldier to the fact that he had been gassed during the war.

The question of a possible causal relationship between exposure to the various war gases and the development of pulmonary tuberculosis has agitated the minds of both the medical profession and the laity from the time of the earliest use of these gases. Both the medical profession and the laity believed at first that soldiers who had been gassed would be especially susceptible to pulmonary tuberculosis. Subsequent facts did not justify this fear. The belief, however, that there is a close causal relationship between gassing and tuberculosis has remained firmly fixed in the minds of the laity and also in the minds of those physicians who have not kept abreast of the times by studying the current medical literature. The literature on the clinical aspects of this subject was recently reviewed by me.[2] The question has been discussed by the medical profession, and, on the basis of clinical observations, physicians are gradually becoming convinced that the belief was not justified. A large number of physicians, working with ex-soldiers who were gassed during the war, have amassed a considerable volume of clinical data. There is overwhelming evidence that gassing does not predispose to pulmonary tuberculosis.

HISTORICAL

No matter how convincing clinical evidence relative to any mooted question may be, the wisdom of applying the test of experimentation

* Submitted for publication, Aug. 16, 1928.

* From the Pathological Section, Medical Research Division, Chemical Warfare Service, Edgewood Arsenal, Edgewood, Md.

1. Koontz, A. R.: When Do Lungs Return to Normal Following Exposure to War Gases? Arch. Int. Med. **36**:204 (Aug.) 1925.

2. Koontz, A. R.: War Gases and Tuberculosis: An Experimental Study, Arch. Int. Med. **39**:833 (June) 1927.

cannot be questioned. The conditions determining the laws of cause and effect can be controlled and studied in experiments on animals in a manner quite impossible with human clinical material. For this reason, several years ago I [2] undertook a series of experiments in an effort to determine what relationship, if any, existed between exposure to high concentrations of toxic gases and the development of pulmonary tuberculosis. Large numbers of rabbits were given varying doses of tubercle bacilli in the vein of the ear, so that the first point of lodgment would be the capillary bed of the lungs. Half of the animals were gassed with mustard gas, phosgene and lewisite at varying times, before and after the injection of the tubercle bacilli; the other half were kept as controls. The experiments clearly demonstrated that the gassed rabbits were not more susceptible to tuberculosis than were the control animals inoculated at the same time with the same dose of bacilli. Indeed, the number of lobes of the lung involved in the gassed animals was slightly less than in the controls. It was further shown that the gassing of animals that already had well developed tuberculosis did not appreciably accelerate the progress of the tuberculous process.

It was noted in these experiments that the difference between the amount of tuberculosis in the animals gassed with lewisite and in the controls was greater than that between the animals gassed with mustard gas and phosgene and their controls. The reason for this fact was believed to be that the animals gassed with lewisite received much smaller doses of tubercle bacilli than those gassed with mustard gas and phosgene; it was easier to detect differences because the infection was not so generalized and not so massive as when larger doses of bacilli were injected. I was, therefore, led to believe that had smaller doses been used throughout the experiments, the results might have shown an even greater difference between the amount of tuberculosis in the gassed animals and in the controls. The results of the experiments with lewisite pointed to an actual inhibiting effect of this gas on the development of pulmonary tuberculosis. They made me wonder whether these impressions would be confirmed by a larger number of experiments, and also whether the same inhibitory influence could be shown to be possessed by the other important gases used in the war.

EXPERIMENTAL WORK

It was determined, therefore, to conduct a more extensive series of experiments, as opportunity presented, dealing separately with each of the principal war gases and using small doses of bacilli in order to determine just what inhibitory effect, if any, they might prove to have on the development of pulmonary tuberculosis.

Mustard gas was the first gas investigated, and the series of experiments conducted with this gas have now been completed. My purpose in this paper is to present the results obtained.

METHOD

In each set of experiments, all animals were inoculated with the same dose of tubercle bacilli per kilogram of body weight. Half of the animals were kept as controls, while the other half were gassed with approximately the lethal concentration of mustard gas. All animals, both controls and gassed animals, were killed from six to ten weeks after inoculation. A notation was made as to the number of cases of tuberculosis in both gassed animals and controls. Each animal having tuberculosis was marked one plus, two plus, three plus or four plus, according to the number of lobes of the lung involved. (The two upper lobes of the right lung were considered as one lobe.) The presence or absence of tuberculosis was determined by both gross and microscopic examination.

Young glycerin-broth cultures of virulent human tubercle bacilli were used in all experiments. Although the cultures used were from the same strain as those used in the experiments reported previously,[2] it was noted that the virulence of the strain had become considerably attenuated, and that larger doses than before were necessary to produce the same amount of tuberculosis.

All rabbits were weighed at the time of inoculation and at autopsy, as in the previous experiments.[2] This has served only to confirm my original conclusions that this information is valueless. Rabbits with tuberculosis gain weight steadily, while others without tuberculosis and kept under exactly the same living conditions, may lose weight, for no accountable reason. The data, therefore, with regard to weight have been left out of the tables.

COMMENT

The results of some of the experiments are shown in the tables; lack of space has prevented the tabulation of all experiments. Tables 1 to 3, inclusive, show in detail the results of typical experiments. Table 4 tabulates the results obtained from all experiments and shows the total number of gassed animals and controls that developed tuberculosis; it also shows the extent of the tuberculous process in the tuberculous animals, as indicated by the number of lobes of the lung involved. Animals that died in less than ten days from the date of inoculation with tubercle bacilli, from the effects of gassing or other causes, are not included in this total, since these animals could not possibly have developed tuberculosis so soon after inoculation. This arbitrary time limit was applied to both the gassed animals and the controls, so as to include only those animals that really had an opportunity to develop tuberculosis. The inclusion of animals that died two or three days after inoculation from irrelevant causes would only have vitiated the results.

In only exceptional instances in this series of experiments was tuberculosis found in organs other than the lungs. This was undoubtedly due to the fact that only small doses of tubercle bacilli were injected, and that the strain had become somewhat attenuated since the experiments first reported [2] were conducted. On account of the small number of cases in which other organs than the lungs were involved, it is not considered worth while to include them in the tables as is would necessitate carrying a practically blank column.

That gassing with mustard gas has an inhibitory effect on the development of pulmonary tuberculosis is clearly shown by a study of these tables. Only 64 per cent of the total number of gassed animals developed tuberculosis; of the total number of controls which were inoculated at the same time with the same dose of bacilli, 83 per cent developed the disease. In the gassed animals that developed tuberculosis, 42 per cent of the possible total number of lobes of the lung were involved, while in the controls that developed tuberculosis, 58 per cent of the possible total number of lobes were involved. Among all the animals that developed

TABLE 1.—*Results Showing the Extent of Tuberculosis in Animals That Were Gassed and in Those That Were Not Gassed*

Number	Dose of Tubercle Bacilli* in Mg. per Kg. of Body Weight	Date Injected†	Result	Time Between Injection and Autopsy, Days	Time of Gassing in Relation to Injection	Lung Involvement
			Gassed Animals			
425	0.005	11/30/26	Killed	46	2 weeks before and 20 hours after	+++‡
426	0.005	11/30/26	Died	18	2 weeks before and 20 hours after	—
427	0.005	11/30/26	Killed	46	2 weeks before and 20 hours after	++++
428	0.005	11/30/26	Killed	46	2 weeks before and 20 hours after	+++
429	0.005	11/30/26	Killed	46	2 weeks before and 20 hours after	—
430	0.005	11/30/26	Killed	46	2 weeks before and 20 hours after	—
			Controls			
431	0.005	11/30/26	Killed	46	.....	++
432	0.005	11/30/26	Killed	46	.....	—
433	0.005	11/30/26	Killed	46	.....	++++
434	0.005	11/30/26	Killed	46	.....	+++
435	0.005	11/30/26	Killed	46	.....	++++
436	0.005	11/30/26	Killed	46	.....	++++

* Twenty-two day old glycerin-broth culture used for inoculations.

† Twenty hours after inoculation, the first six animals in the table were gassed with mustard gas (concentration of 0.0152 mg. per liter of air) for one hour. These animals had also been gassed with mustard gas in a field test, two weeks previous to inoculation, concentration of gas and time of exposure unknown.

‡ The plus marks indicate the number of lobes involved.

tuberculosis, therefore, the extent of the tuberculous process was greater in the nongassed than in the gassed animals.

These results are in accord with the experience of Francine [3] who did not find an increase in tuberculosis in necropsies on soldiers who had been gassed. They are also in accord with the known clinical fact that tuberculosis is rare in persons who have mitral stenosis. Apparently, congestion of the lungs is a condition unfavorable to the growth of the tubercle bacillus. The congestion produced by mustard gas may be the factor responsible for its inhibitory effect on tuberculosis. This seems to me more probable than that there is a beneficial bactericidal action on

3. Gilchrist, H. L.: Warfare Gas and Tuberculosis; Testimony of Dr. Albert P. Francine Before the Senate Committee, Mil. Surgeon **54**:470 (April) 1924.

TABLE 2.—*Results Showing the Extent of Tuberculosis in Animals That Were Gassed and in Those That Were Not Gassed*

Number	Dose of Tubercle Bacilli* in Mg. per Kg. of Body Weight	Date Injected†	Result	Time Between Injection and Autopsy, Days	Time of Gassing in Relation to Injection	Lung Involvement
			Gassed Animals			
449	0.005	12/29/26	Killed	49	1 day before	+‡
450	0.005	12/29/26	Killed	49	1 day before	—
451	0.005	12/29/26	Killed	49	1 day before	—
452	0.005	12/29/26	Killed	49	1 day before	—
453	0.005	12/29/26	Killed	49	1 day before	—
454	0.005	12/29/26	Killed	49	1 day before	—
			Controls			
455	0.005	12/29/26	Killed	49	.....	++++
456	0.005	12/29/26	Killed	49	.....	++++
457	0.005	12/29/26	Killed	49	.....	++
458	0.005	12/29/26	Killed	49	.....	++
459	0.005	12/29/26	Killed	49	.....	—
460	0.005	12/29/26	Killed	49	.....	++++

* Seven day old glycerin-broth culture used for inoculations.
† One day before inoculation, the first six animals in the table were gassed with mustard gas (concentration of 0.0133 mg. per liter of air) for one hour.
‡ The plus marks indicate the number of lobes involved.

TABLE 3.—*Results Showing the Extent of Tuberculosis in Animals That Were Gassed and in Those That Were Not Gassed*

Number	Dose of Tubercle Bacilli* in Mg. per Kg. of Body Weight	Date Injected†	Result	Time Between Injection and Autopsy, Days	Time of Gassing in Relation to Injection	Lung Involvement
			Gassed Animals			
509	0.01	4/11/27	Killed	51	1 day after	++++‡
510	0.01	4/11/27	Killed	51	1 day after	+
511	0.01	4/11/27	Killed	51	1 day after	++++
512	0.01	4/11/27	Killed	51	1 day after	—
513	0.01	4/11/27	Killed	51	1 day after	++++
514	0.01	4/11/27	Killed	51	1 day after	—
			Controls			
515	0.01	4/11/27	Killed	51	.....	++++
516	0.01	4/11/27	Died	22	.....	++++
517	0.01	4/11/27	Killed	51	.....	++
518	0.01	4/11/27	Killed	51	.....	++++
519	0.01	4/11/27	Killed	51	.....	++++
520	0.01	4/11/27	Killed	51	.....	+

* Twelve day old glycerin-broth culture, not growing very well, used for inoculations.
† One day after inoculation, the first six animals in the table were gassed with mustard gas (concentration of 0.0174 mg. per liter of air) for one hour.
‡ The plus marks indicate the number of lobes involved.

TABLE 4.—*Tabulation of Total Results from all Experiments*

	Total Number of Animals	Number of Animals Developing Tuberculosis	Percentage of Animals Developing Tuberculosis	Total Number of Lung Lobes Involved	Percentage (of Possible Total) of Lung Lobes Involved
Gassed animals.....	78	50	64	130	42
Controls.....	77	64	83	178	58

the part of the gas. Corper and Rensch,[4] however, have reported that mustard gas is bactericidal for virulent human tubercle bacilli in vitro, and that when injected with bacilli into guinea-pigs, it distinctly retards the development of tuberculosis in these animals.

SUMMARY AND CONCLUSIONS

One hundred and sixty-eight rabbits were inoculated in the vein of the ear with virulent human tubercle bacilli. Half of the animals were gassed for one hour with approximately the lethal dose of mustard gas; the other half were kept as controls. From six to ten weeks after their inoculation, all the animals were killed, autopsies performed, and the viscera studied for tuberculous lesions. Sixty-four per cent of the animals that had been gassed had pulmonary tuberculosis; 83 per cent of the controls had pulmonary tuberculosis. The tuberculous process was much more extensive in the control animals that developed the disease than in the gassed animals that contracted it.

These experiments clearly demonstrate that gassing with mustard gas has an inhibitory effect on the development of pulmonary tuberculosis in rabbits.

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4. Corper, H. J., and Rensch, O. B.: The Effect of Mustard Gas (Dichlorethylsulphide) on Experimental Tuberculosis, J. Infect. Dis. **28**:286 (March) 1921.

NORMAL BLOOD DETERMINATIONS IN THE SOUTH *

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The opinion has been frequently expressed that the red cell count and particularly hemoglobin are low in the South. Musser and Wirth [1] advanced the thesis that anemia is more prevalent in the South than the North. Lippincott [2] concluded that the normal average percentage of hemoglobin and erythrocyte counts in the region of the state of Mississippi are lower than the usually accepted standards. His determinations, however, were made on patients in hospitals and clinics, and his methods do not appear to have been accurate.

On the other hand, Major W. P. Chamberlain,[3] studying the blood of a large number of soldiers in the Philippines, found a normal count for red corpuscles and possibly a slightly lowered precentage of hemoglobin. Like Chamberlain, Balfour [4] expressed the belief that climate alone does not produce any change in the blood and that so-called tropical anemia is apparent rather than genuine.

In order to determine the accuracy of these statements, specimens of the blood of 100 healthy young men have been examined by the most accurate methods available. An excellent basis for comparison is furnished by the work of Osgood,[5] who reported red cell counts, hemoglobin and determination of cell volume on 137 young men living on the Western coast of the United States. In order to make comparison easier, our methods have been similar to those described by Osgood.

We have been further stimulated in our work by the need for a large series of accurate, normal examinations of the blood, made in different parts of the world, in order that accurate, normal figures on the blood may be computed. Few are aware that the figures at present accepted as normal are based on a small number of determinations carried out

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1. Musser, J. H., and Wirth, Willard R.: Anemia in the South, Ann. Clin. Med. **5**:861 (March) 1927.

2. Lippincott, L. S.: Hemoglobin and Erythrocytes in the South, J. Lab. & Clin. Med. **12**:679 (April) 1927.

3. Chamberlain, W. P., quoted by H. Sewall: Oxford Medicine, vol. 1, New York, 1927, p. 491.

4. Balfour, Andrew: Effects of Tropical Climates, in Byam and Archibald's The Practice of Medicine in the Tropics, vol. 1, London, Henry Frowde & Hodder and Stoughton, 1921, p. 4.

5. Osgood, Edwin E.: Hemoglobin, Color Index, Saturation Index and Volume Index Standards in Men, Arch. Int. Med. **37**:685 (May) 1926.

by inaccurate and obsolete methods. Bie and Möller [6] stated that the figures for normal red cell counts (5,000,000 in men and 4,500,000 in women) are based on the work of Vierorde (1852) and Welcher (1854), each of whom examined only two subjects. Likewise, the variety of standards accepted as 100 per cent by the various hemoglobinometers reflects the inaccurate foundation on which they depend.

SUBJECTS EXAMINED

The subjects of our examination were normal, healthy students between the ages of 20 and 30 years. Each of these students has been the subject of a thorough laboratory examination during the course of the academic session, including urinalysis, renal function tests, gastric analysis, several examinations of the stools and even chemical examination of the blood. The negative results of these examinations, along with a negative history, give us reason to believe that they were all free of organic disease. A few of them were slightly "under par," judging by their height-weight ratio, and this was shown in their blood count. They were, however, free of organic disease. Eighty per cent were Americans born of American parents; the remainder were of French, Spanish, Italian or Russian descent. All but 5 per cent were born in the United States. The majority have resided in the South (Louisiana, Mississippi, Alabama, Texas, North Carolina, Florida, Oklahoma and Arkansas) most of their lives. With the exception of a few who have been North during the summer vacation, all have resided continuously in this locality for the past three years. The examinations were made during May and June (before, during and after college examinations). During the period of examination, the atmospheric temperature ranged approximately between 75 and 88 or 90 F.

Many of the specimens of blood were drawn about noon, before and after lunch. Others were drawn about 9 a. m. In this way, any possible effect of a diurnal variation of hemoglobin has been obviated.

METHODS

Ten cubic centimeters of venous blood were drawn into a syringe containing 40 mg. of finely powdered neutral potassium oxalate, and thoroughly mixed. The blood so obtained was used for red cell counts, hemoglobin estimation and "blood volume" determinations. The amount of oxalate used was found to have no effect on cell count or hemoglobin. Its effect on blood volume is discussed under that heading.

Red Cell Counts.—Bass-Johns counting chambers and pipets (certified by the United States Bureau of Standards) were used. Before using any sample of blood, care was taken to mix it thoroughly in order to avoid any error resulting from sedimentation. Fresh Toison's solution was used for dilution and at least two dilutions of 1:200 were counted in each case. Only results agreeing within

6. Bie, V., and Möller, P.: Constitution du sang humain normal, Arch. de mal. du coeur, **15**:177 (April) 1922.

100,000 cells were accepted, the average of two such closely agreeing figures being recorded. A number of red cell counts were made to determine any possible difference resulting from the use of venous blood as compared with blood obtained by finger puncture. Whenever care was taken to secure a free flow of blood from the finger (in order to prevent dilution resulting from the forcing out of serum in compressing the finger), counts made on blood obtained from the latter source were as high as those made on venous blood.

TABLE 1.—*Hemoglobin Estimations by Newcomer and Van Slyke Methods*

	Hemoglobin		
	Newcomer		Van Slyke
Blood Number	Per Cent	Gm.	Gm.
1	100.3	16.97	18.16
2	95.3	16.12	17.46
3	92.0	15.57	16.99
4	78.0	13.20	15.08
5	70.9	12.00	14.19
6	69.3	11.73	13.82
7	63.8	10.80	13.58
8	61.8	10.46	12.40
9	53.7	9.09	10.58
10	53.0	8.97	10.48

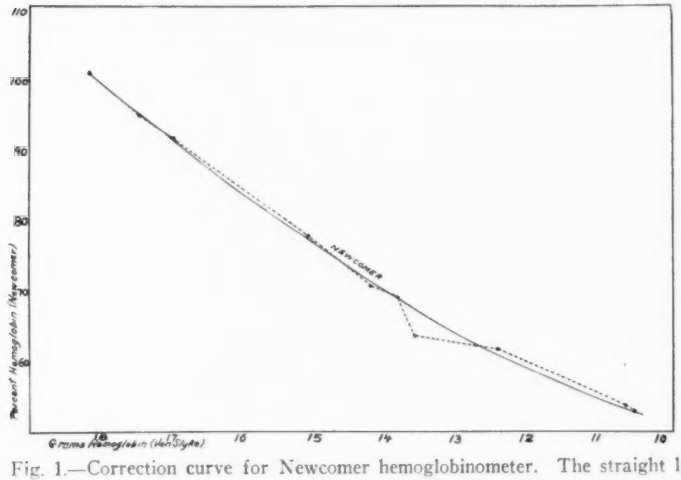

Fig. 1.—Correction curve for Newcomer hemoglobinometer. The straight line represents the correction curve and the dotted line represents the readings.

Hemoglobin Estimation.—Prior to the commencement of our work on students, the accuracy of the generally used clinical methods of hemoglobin estimation (Sahli, Newcomer, Dare, Tallqvist) was tested, readings on the various instruments being compared with those obtained by van Slyke's method of determining the oxygen capacity of the blood.[7] All the instruments examined proved to be inaccurate. Readings on the Newcomer instrument, however, although lower than those obtained by the van Slyke method, proved to be consistently lower (table 1), so that it was found possible to plot a correction curve for our

7. Van Slyke, D. D., and Stadie, W. C.: The Determination of Gases of the Blood, J. Biol. Chem. **49**:1 (Nov.) 1921.

Newcomer instrument (fig. 1). As this instrument is readily available for clinical use and apparently accurate when corrected, it was chosen for our work. Readings were not made until the acid hematin solution was allowed to stand for one-half hour. A time correction according to the formula worked out by Newcomer [8] was made in each case.

Cell Volume.—Volume determinations were made by centrifugalization of 4 cc. of blood at 3,500 revolutions per minute. After an initial centrifugalization for from twenty to thirty minutes, readings were made at five minute intervals until the red cell volume did not show any change at two successive readings. The centrifuge tube was made by sealing off the end of a 10 cc. Mohr pipet and cutting it off a little above the 6 mark. It was carefully recalibrated.

RED CELL COUNTS IN NORMAL MALES

Red cell counts made with reasonably accurate care have been reported for 178 men between the ages of 19 and 30 years: Bierring [9] (Sweden) reported four men, averaging 5,050,000; Bie and Möller [6] (Denmark), ten men, averaging 5,530,000; Gram and Norgaard [10]

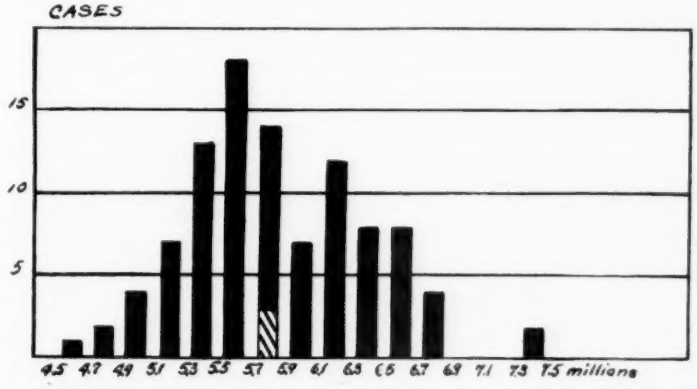

Fig. 2.—Red cell counts in 100 healthy young men. The average occurs in columns with shaded lower portion.

(Denmark), seven men, averaging 5,390,000; Haden [11] (Kansas City), twenty men, averaging 5,080,000; Osgood [5] (Oregon), 137 men, averaging 5,390,000, making a total of 178 men, averaging 5,410,000.

8. Newcomer, H. S.: A New Optical Instrument for the Determination of Hemoglobin, J. Biol. Chem. **55**:569 (April) 1923.

9. Bierring, K.: Svingninger i erythrocyttallet hos normale mennesker, Ugesk. f. Laeger **82**:1445 (Nov. 18) 1920, quoted by Osgood.

10. Gram, H. C., and Norgaard, A.: Relation between Hemoglobin, Cell Count and Cell Volume in the Venous Blood of Normal Human Subjects, Arch. Int. Med. **31**:164 (Feb.) 1923.

11. Haden, R. L.: Accurate Criteria for Differentiating Anemias, Arch. Int. Med. **31**:765 (May) 1927.

A number of other counts which have been reported by other authors are tabulated by Bie and Möller [6] and by Osgood,[5] but none of these are sufficiently accurate to be included with the foregoing data.

Our average in 100 men between the ages of 19 and 30 years was 5,850,000. Figure 2 shows that 95 per cent of our counts ranged between 5,000,000 and 6,900,000, while 64 per cent ranged between 5,300,000 and 6,300,000. Two students had counts above 7,000,000, while five had counts below 5,000,000. Neither of the former showed any clinical evidence of polycythemia, while all of the latter were slightly underweight although not suffering from any disease. All of these figures were included in computing our average.

Osgood, working in Oregon, found 90 per cent of the counts between 4,700,000 and 6,100,000. It is noteworthy that 36 per cent of our students had counts above 6,000,000, while only 7.3 per cent of Osgood's cases were above that figure.

When our figure of 5,850,000 is included with the 178 accurate counts already quoted, the average for the 278 persons is 5,570,000.

HEMOGLOBIN CONTENT OF THE BLOOD OF NORMAL MALES

A statement of the inaccurate foundation on which our methods of hemoglobin estimation (in themselves inexact) are based cannot be too often repeated. It is not generally known that the Sahli instrument accepts 17.2 Gm. per hundred cubic centimeters of blood as 100 per cent, while the Dare is based on 13.8 Gm. as the equivalent of 100 per cent; the Newcomer standard is 16.92 Gm., whereas the Tallqvist scale is set on a basis of 15.8 Gm. This wide range in the accepted standard is merely a reflection of the inaccurate basis on which these figures have been calculated. As a matter of fact, until Osgood[12] published his work, a sufficient number of accurate determinations on which to calculate a standard was not available. When one considers that hemoglobin varies not only with age, sex [13] and time of day,[14] but also with altitude and perhaps climate and race, it is obvious that, for example, Haldane's [15]

12. Osgood, Edwin E., and Haskins, Howard D.: Relation between Cell Count, Cell Volume and Hemoglobin Content of Venous Blood of Normal Young Women, Arch. Int. Med. **39**:643 (May) 1927. Osgood (footnote 5).

13. Williamson, C. S.: Influence of Age and Sex on Hemoglobin, Arch. Int. Med. **18**:505 (Oct.) 1916.

14. Ward, H. C.: The Hourly Variations in the Quantity of Hemoglobin and in the Number of Corpuscles in Human Blood, Am. J. Physiol. **11**:394 (July) 1904. Dreyer, G.; Bazett, H. C., and Pierce, H. F.: Diurnal Variation in the Hemoglobin Content of the Blood, Lancet **199**:588 (Sept. 18) 1920. Rabinowitch, I. M.: Variation of Percentage of Hemoglobin in Man During Day, J. Lab. & Clin. Med. **9**:120 (Nov.) 1923.

15. Haldane, J.: The Colorimetric Determination of Hemoglobin, J. Physiol. **26**:497 (June) 1901.

average of 13.8 Gm. in twelve men in England (determined by a method that is now obsolete) cannot be taken as the basis for 100 per cent all over the world.

Reliable hemoglobin estimations on healthy males between the ages of 19 and 30 years have been reported as follows: Williamson [13] (spectrophotometer), thirty-six men, averaging 16.8 Gm.; Bie and Möller [6] (Meisling colorimeter, checked by oxygen apparatus of Peterson-Bohr), ten men, averaging 14.75 Gm.; Gram and Norgaard [10] (Autenreith-Königsberger colorimeter, corrected by oxygen method), seven men, averaging 14.89 Gm.; Haden [11] (van Slyke's method), twenty men,

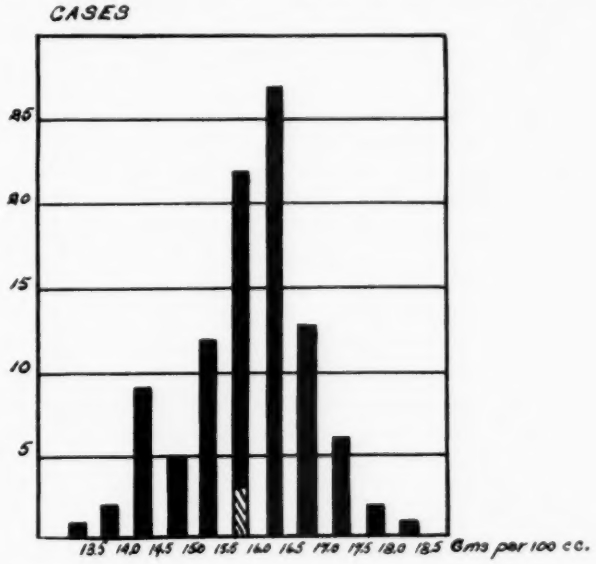

Fig. 3.—Total hemoglobin in 100 healthy young men.

averaging 15.83 Gm.; Brown and Rowntree [16] (Palmer's method, checked by Osgood-Haskin's method [17]), seven men, averaging 16.27 Gm.; Osgood [5] (Osgood-Haskins' method [17]), 137 men, averaging 15.76 Gm., making a total of 217 men, averaging 15.88 Gm.

This average agrees closely with our average of 15.87 Gm. in 100 men. The average for the total of 317 estimations is 15.88 Gm. The

16. Brown, G. E., and Rowntree, L. G.: The Volume and Composition of the Blood and the Changes Incident to Diuresis in Cases of Edema, Arch. Int. Med. **35**:129 (Jan.) 1925.

17. Estimations of hemoglobin by the Osgood-Haskins' method compare very closely to those obtained by the van Slyke method according to Osgood.

extremes in 171 of the foregoing 217 estimations were 13.3 and 19.46 Gm. while our extremes were 13.4 and 18.3 Gm.

Seventy-three per cent of our determinations ranged between 15 and 16.9 Gm. (fig. 3), while 87 per cent were between 14 and 16.9 Gm., with only 10 per cent above that figure. It is interesting to note that 85 per cent of Osgood's [5] 137 estimations occurred between 14 and 16.9 Gm., with also 10 per cent above that figure.

COLOR INDEX

As color index is an expression of the relation of hemoglobin to the number of erythrocytes—the percentage of hemoglobin in proportion to the percentage of cells—it is important that the hemoglobin figure taken as 100 per cent be calculated to a basis of 5,000,000 red cells, since that is the number of cells taken as 100 per cent in the calculation. Thus, if a study of normal figures should reveal that the average hemoglobin figure is 16.5 Gm. in blood samples which on the average contain 5,500,000 red cells, then 15 Gm. (16.5 calculated to a count of 5,000,000) should be taken as the 100 per cent on which percentage of hemoglobin used in the determination of color index is based. For the expression, "the number of grams of hemoglobin per 100 cc. of blood calculated to a red cell count of 5 million per cu. mm.," Osgood [5] has suggested the term "hemoglobin coefficient." Before accurate color indexes can be calculated the normal average hemoglobin coefficient must be determined. It is this average hemoglobin coefficient, calculated on the basis of a large series of accurate determinations of hemoglobin and red cell counts, that should be used as 100 per cent by the various clinical hemoglobinometers.

We have been able to find only the following satisfactory data for the calculation of a standard hemoglobin coefficient for men between the ages of 19 and 30: Bie and Möller,[6] ten men, averaging 13.34 Gm.; Gram and Norgaard,[10] seven men, averaging 13.82 Gm.; Haden,[11] twenty men, averaging 15.57 Gm.; Osgood,[5] 137 men, averaging 14.66 Gm., making a total of 174 men, averaging 14.65 Gm.

Our average of 13.66 Gm. in 100 men is considerably lower than that found by Haden or Osgood and corresponds to the observations of Gram and Norgaard and of Bie and Möller. Our low hemoglobin coefficient is the outcome of our higher average red cell count, as our average hemoglobin figure is practically the same as that found by Haden and by Osgood.

The hemoglobin coefficient in 174 cases under our observations ranged between 12.32 and 18.63 Gm. Osgood found 90 per cent to occur between 13 and 16.5 Gm. Our coefficients ranged from 10.4 to 17.4

Gm., with 90 per cent between 12 and 16 Gm. Frequency figure 4 shows a definite peak in the region of the average.

The average of all these 274 determinations is 14.29 Gm. As this figure represents a wider range than that on which Osgood's hemoglobin coefficient (14.7) is based, we suggest that 14.3 Gm. be accepted as the hemoglobin coefficient for normal men between the ages of 19 and 30 years. Recalculation of color indexes on this basis shows the average indexes to be as follows: Haden (Kansas City), twenty men, averaging 1.09; Osgood (Oregon), 137 men, averaging 1.02; Gram and Norgaard (Denmark), seven men averaging 0.97; Wintrobe and Miller (Louisiana), 100 men, averaging 0.95; Bie and Möller (Denmark), ten men, averaging 0.93, making a total of 274 men, averaging 1.

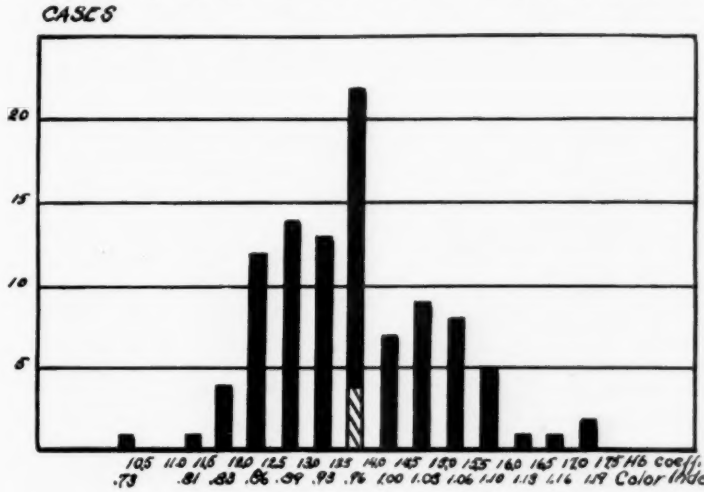

Fig. 4.—Hemoglobin coefficients and color indexes in 100 healthy young men.

Although the difference between these color indexes is relatively small, one is led to wonder whether or not the effect of climate is evidenced by this slight variation (fig. 5).

TOTAL CELL VOLUME

In collecting venous blood for the determination of its red cell count, hemoglobin content and cell volume, it is important that a method be employed which leaves the blood undiluted and isotonic. The addition of heparin or hirudin fulfils these requirements, but has the disadvantage of being very expensive. When dry neutral potassium oxalate is used, the blood remains undiluted but a somewhat hypertonic solution is produced.

A number of experiments were carried out by the following method to determine the error resulting from the use of such a hypertonic solution: To 30 cc. of blood, obtained by venous puncture, sufficient heparin was added to prevent clotting. Ten cubic centimeters of this

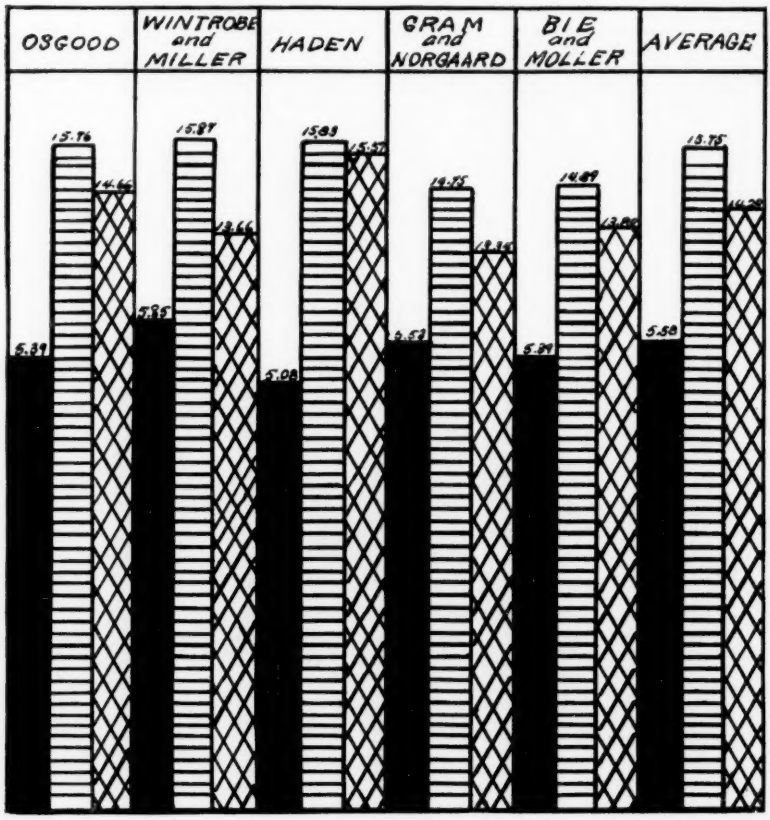

Fig. 5.—Cell counts and hemoglobin in different parts of the world on healthy young men. The black areas represent the red blood cells; the lined areas, the hemoglobin, and the cross-patched areas, the hemoglobin coefficient.

blood was set aside as a control. To another 10 cubic centimeters, 40 mg. of neutral potassium oxalate was added and thoroughly mixed, while the remaining 10 cc. was mixed with 20 mg. of potassium oxalate. Red cell counts, hemoglobin and volume determinations were then carried out on each of these three samples by our usual methods.

Cell counts and hemoglobin values did not show an appreciable difference in any of the samples. A definite decrease of cell volume, however, was found to result from the production of a hypertonic solution.

Observations on the cell volume in two experiments are shown in table 2.

Our experiments showed that the addition of 20 mg. of neutral potassium oxalate to 10 cc. of blood (the proportion used by Osgood) caused an average shrinkage of cell volume of 3.68 per cent. This tallies closely with Osgood's figure of 3.5 per cent. The addition of 40 mg. of oxalate to 10 cc. of blood (the proportion employed in all our work) caused an average shrinkage of 6.7 per cent. As the heparin method is theoretically ideal, all of our volume figures have been corrected by adding 6.7 per cent.

Haden [11] reported volume determinations on twenty men between the ages of 19 and 30 years. He used 1.6 per cent sodium oxalate

TABLE 2.—*Effect of Oxalate on Cell Volume*

	Experiment 1		Experiment 2	
	Per Cent of Cell Volume	Per Cent of Difference	Per Cent of Cell Volume	Per Cent of Difference
Heparinized blood without oxalate	49.7	0	49.2	0
Heparinized blood with 20 mg. oxalate added to 10 cc.	48.0	3.54	47.4	3.83
Heparinized blood with 40 mg. oxalate added to 10 cc.	46.7	6.42	46.0	6.98

solution and centrifugated at 2,500 revolutions per minute for one-half hour. As sometimes centrifugation to constant volume is not complete at the end of thirty minutes, some of his figures may be high. His average was 46.5 cc. of cells per hundred cubic centimeters of blood. Bie and Möller [6] used defibrinated blood and were careful to centifugate to constant volume. Their average was 46.4 cc. Gram and Norgaard,[10] using the hirudin method, and centrifugating for one and a half hours at 3,000 revolutions per minute, found an average volume of 45.9 cc. in seven men. Osgood,[5] using the oxalate method, found an average of 44.84 cc. in ninety-four men. The addition of 3.5 per cent to this figure, to allow for shrinkage resulting from the use of 20 mg. oxalate with 10 cc. of blood, brings it to 46.4 cc. Whether, on account of their methods being a little questionable, the observations of Haden and of Bie and Möller be included, or not, the average cell volume, corrected to a basis of the figure obtained when the blood used is undiluted and isotonic, is 46.4 cc. With this figure our average of 46.5 cc., in 100 men, agrees closely. The average of the total of 231 determinations is 46.4 cc.

Frequency figure 6 shows that 85 per cent of our results ranged between 40 and 50 cc., with 54 per cent between 46 and 50 cc. This agrees closely with Osgood's figure of over 90 per cent between 40 and 50 cc. and 56 per cent between 45 and 49 cc.

VOLUME INDEX

Capps,[18] in 1903, introduced the term "volume index" to express the cell volume of a given sample of blood as compared with the normal. Its determination is similar to that of the color index with the difference that cell volume percentage is considered instead of hemoglobin percentage. A volume index of more than 1 is always present when the color index is greater than 1, and also in many instances in which the color index is less than 1. The volume index is not only of greater

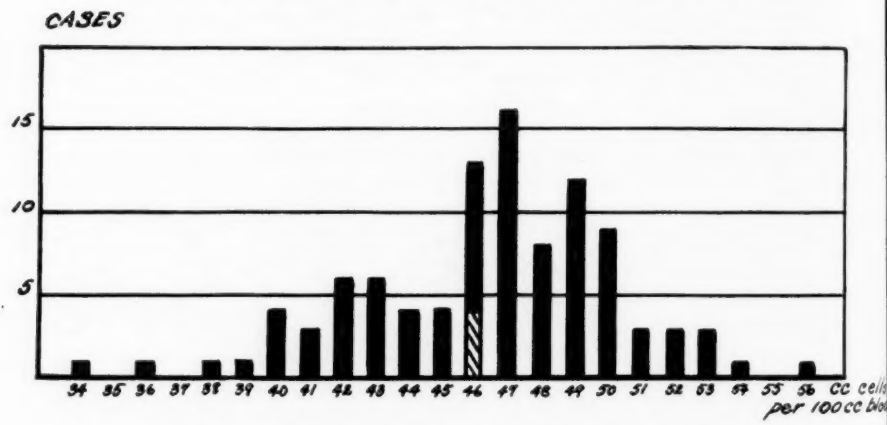

Fig. 6.—Total volume of cells in 100 healthy young men.

value than the color index, but serves as an important check on it. As an accurate determination of cell volume is relatively easy to carry out, volume index determinations are of particular value as long as the generally used methods of hemoglobin determination remain as inaccurate as they now are.

The volume index is determined by dividing the percentage volume of cells (expressed as a percentage of the average normal volume for the given sex and age group and calculated to a count of 5,000,000 red cells) by the percentage of red cells (5,000,000 being 100 per cent). Osgood [5] has coined the term "volume coefficient" as a substitute for the expression, "volume of packed red cells per 100 cc. of blood calculated to a red cell count of 5 million."

18. Capps, J. A.: A Study of Volume Index, J. M. Research **5**:367, 1903.

Accurate data for the determination of volume coefficient are supplied by Gram and Norgaard,[10] whose average was 42.2 in seven men, and by Osgood,[5] whose average becomes 42.23 in ninety-four men, when a correction is made for the error resulting from the use of oxalate. The average of these 101 determinations is 42.23 cc. Our average (corrected for the use of oxalate) was 39.95 cc. in 100 men. Our low volume coefficient is the result of our high average red cell count. The average for these 201 determinations is 41.09 cc. This agrees with the figure (41 cc.) originally suggested by Osgood as the normal volume coefficient for young men between the ages of 19 to 30 years.

Osgood does not consider the data given by Haden[11] (average volume coefficient of 45.8 in twenty men) or by Bie and Möller[6] (average of 42.0 in ten men) as being sufficiently accurate to be included. In any case, the addition of their observations does not greatly increase

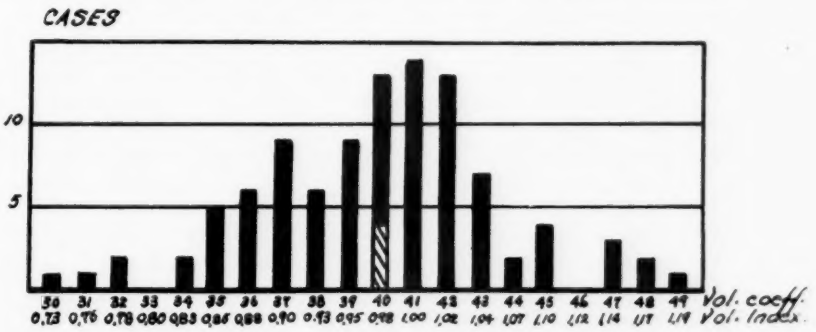

Fig. 7.—Volume indexes and volume coefficients in 100 healthy young men.

the average volume coefficient (the average for 231 men becomes 41.54 cc.).

Recalculation of average volume indexes from the data supplied by various authors, on the basis of a volume coefficient of 41 cc., reveals the following: Haden (Kansas City), twenty men, averaging 1.12; Osgood (Oregon), ninety-four men, averaging 1.05; Gram and Norgaard (Denmark), seven men, averaging 1.04; Bie and Möller (Denmark), ten men, averaging 1.02; Wintrobe and Miller (Louisiana), 100 men, averaging 0.97, making a total of 231 men, averaging 1.02.

Frequency figure 7 shows that our most frequently occurring volume coefficient was 41, corresponding to a volume index of 1. Our average was 39.7, corresponding to a volume index of 0.97. Seventy-seven per cent of our volume coefficients ranged between 37 and 45 cc., corresponding to volume indexes of 0.90 to 1.1, while 88 per cent fell between 35 and 45 cc., corresponding to volume indexes of 0.85 to 1.1. Osgood found 90 per cent between 37 and 45 cc., corresponding to volume

indexes of 0.9 to 1.1 and 98 per cent between 35 and 45 cc., corresponding to volume indexes of 0.85 to 1.1.

SATURATION INDEX

It is a common impression that color index is an expression of the saturation of red cell with hemoglobin. This is of course not true, since color index is the expression of the relation of hemoglobin to the number of erythrocytes. To denote the relation of hemoglobin to volume of erythrocytes, Haden [11] has suggested a new index, the "saturation index," which expresses the ratio between the concentration of hemoglobin per unit volume of cells in a particular subject, and the average

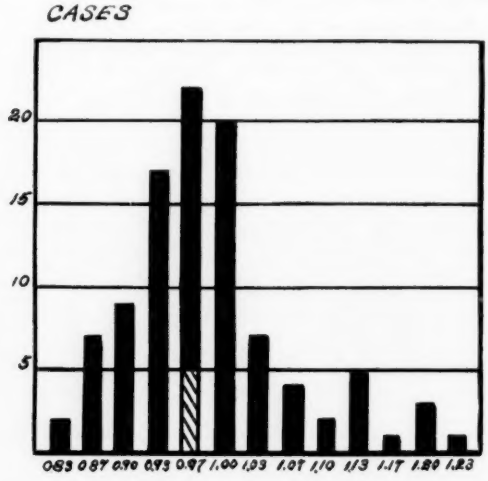

Fig. 8.—Saturation indexes in 100 healthy young men.

concentration of hemoglobin per unit volume of cells in normal subjects of the same sex and age group.

Haden calculated the saturation index by dividing the color index by the volume index. Since this is the same as dividing the hemoglobin percentage by the volume percentage, and since in so doing one derives a clearer conception of the meaning of the saturation index, we suggest that the saturation index be defined as, "the proportion of hemoglobin per unit volume of cells, expressed in proportion to the normal," and be calculated by dividing hemoglobin per cent by volume per cent.

Only in normal blood, in which both the number and the volume of red cells are normal, are color index and saturation index synonomous. According to Haden, a supersaturation of red cells never occurs, and the saturation index never rises above 1.

The available data for normal men between the ages of 19 and 30 years, recalculated on the basis of a hemoglobin coefficient of 14.3 Gm. and a volume coefficient of 41 cc., show the following average saturation indexes: Bie and Möller,[6] ten men, averaging 0.91; Gram and Norgaard,[10] seven men, averaging 0.93; Haden,[11] twenty men, averaging

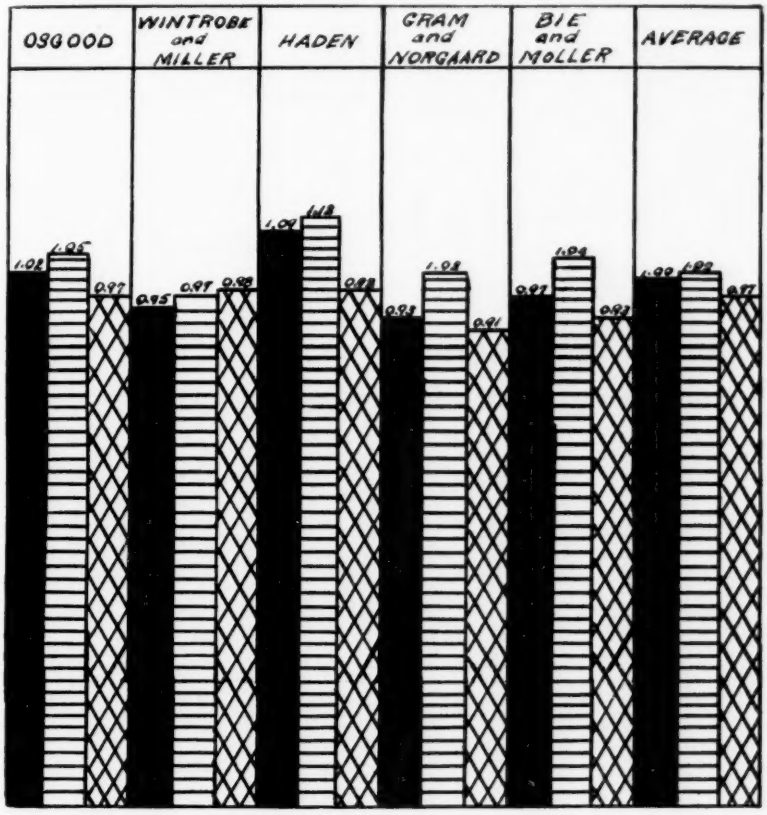

Fig. 9.—Indexes in different parts of the world on healthy young men. The black areas represent the color index; the lined area represents the volume index, and the cross-patched area the saturation index.

0.98; Osgood,[5] ninety-four men, averaging 0.97, making a total of 131 men, averaging 0.97.

Our average for 100 men was 0.98. The average for the 231 men is 0.97.

Frequency figure 8 shows the frequency distribution of saturation indexes in our 100 men. It will be seen that the average and peak coincide. Eighty-one per cent of our saturation indexes occurred between 0.9 and 1.1, as compared with over 90 per cent of Osgood's saturation indexes falling within that range.

The method of determining the color, volume and saturation indexes is best illustrated by an example. A given sample of the blood of a man, aged 25, contains 5,850,000 red cells per cubic millimeter, 15.87 Gm. of hemoglobin per hundred cubic centimeters, and 46.5 cc. packed red cells per hundred cubic centimeters.

The percentage of hemoglobin is 15.87 divided by 14.3 (the hemoglobin coefficient for the given age group and sex) or $\frac{15.87}{14.3} \times 100 = 111$ per cent.

The percentage of cells is $\frac{5.85}{5.0} \times 100 = 117$ per cent.

Color index is $\frac{\text{Hb.\%}}{\text{RBC\%}}$, or $\frac{111}{117} = 0.95$.

The cell volume percentage is 46.5 divided by 41 (volume coefficient for the given age and sex), or $\frac{46.5}{41} \times 100 = 113.4$ per cent.

Volume index is $\frac{\text{Vol.\%}}{\text{RBC\%}}$, or $\frac{113.4}{117.0} = 0.97$.

Saturation index is $\frac{\text{Hb.\%}}{\text{Vol.\%}}$, or $\frac{111.0}{113.4} = 0.98$.

TABLE 3.—*Observations on the Blood in Healthy Young Men Between 19 and 30 Years of Age in Different Parts of the World**

Author	Cases: No.	Cases: Location	Red Blood Cells in Millions	Hemoglobin: Gm.	Hemoglobin: Coefficient	Blood Volume: Red Blood Cells per 100 Cc.	Blood Volume: Coefficient	Indexes: Color	Indexes: Volume	Indexes: Saturation
Osgood	137	Oregon	5.39	15.76	14.60	46.4	42.2	1.02	1.05	0.97
Wintrobe and Miller	100	Louisiana	5.85	15.87	13.66	46.5	39.7	0.95	0.97	0.98
Haden	20	Kansas City	5.08	15.83	15.57	46.5	45.8	1.09	1.12	0.98
Bie and Möller	10	Denmark	5.53	14.75	13.34	46.4	42.0	0.93	1.02	0.91
Gram and Norgaard	7	Denmark	5.39	14.89	13.82	45.9	42.2	0.97	1.04	0.93
Average			5.58	15.75	14.29	46.4	41.5	1.00	1.02	0.97

* Calculations are based on a hemoglobin coefficient of 14.3 Gm. and a volume coefficient of 41 cc.

TABLE 4.—*Observations on the Blood in One Hundred Normal Young Men*

Subject	Age	Red Cell Count, Millions per C.Mm.	Hemoglobin, Gm. per 100 Cc.	Hemoglobin per 100 Cc. per 5 Million Cells	Color Index	Volume of Cells per 100 Cc.	Volume of Cells per 100 Cc. per 5 Million	Volume Index	Saturation Index
1	19	5.48	13.40	12.30	0.86	45.9	41.9	1.02	0.84
2	24	5.09	14.50	12.70	0.89	34.1	30.0	0.73	1.22
3	22	5.59	15.25	13.64	0.96	41.5	37.1	0.91	1.04
4	24	6.61	16.20	12.30	0.86	49.7	37.6	0.92	0.94
5	21	6.09	16.05	13.50	0.93	50.1	41.2	1.00	0.93
6	22	5.54	16.60	15.00	1.05	47.9	43.2	1.05	1.00
7	23	6.03	16.20	13.40	0.94	47.6	39.5	0.97	0.97
8	27	5.20	16.20	15.60	1.09	46.9	45.1	1.10	0.99
9	27	4.68	15.93	17.06	1.18	40.2	42.9	1.05	1.12
10	28	5.88	16.05	13.65	0.95	45.8	39.0	0.95	1.00
11	23	5.40	16.75	15.50	1.09	48.4	44.8	1.08	1.01
12	22	5.53	18.30	16.50	1.16	46.6	42.2	1.02	1.12
13	22	5.32	16.75	15.70	1.10	51.5	48.4	1.18	0.93
14	24	4.70	16.33	17.40	1.21	36.4	38.7	0.95	1.26
15	22	5.84	17.02	14.50	1.02	40.2	34.5	0.84	1.20
16	24	5.32	16.90	15.90	1.11	45.3	42.7	1.03	1.08
17	21	5.37	16.05	15.00	1.04	38.9	36.2	0.89	1.16
18	20	5.58	17.25	15.40	1.08	43.5	39.0	0.95	1.13
19	25	6.53	17.25	13.20	0.92	48.1	36.8	0.90	1.02
20	25	5.14	15.65	15.20	1.06	43.3	42.1	1.03	1.02
21	23	5.64	14.80	13.10	0.92	47.9	42.5	1.03	0.88
22	21	5.02	16.00	14.20	1.00	45.9	40.8	1.00	1.00
23	22	5.99	15.95	13.30	0.93	37.9	31.7	0.77	1.20
24	25	6.15	16.60	13.50	0.94	47.2	38.4	0.94	1.00
25	21	6.67	16.35	12.30	0.86	43.2	31.9	0.79	1.09
26	21	5.46	16.25	14.90	1.04	49.1	44.9	1.09	0.95
27	23	5.27	15.80	15.00	1.05	49.0	46.6	1.13	0.92
28	23	4.98	16.05	16.30	1.13	47.1	47.3	1.15	0.98
29	23	5.18	15.24	14.70	1.03	50.5	48.8	1.19	0.87
30	21	6.09	16.20	12.10	0.85	46.8	35.0	0.85	1.00
31	21	5.58	16.05	14.56	1.00	46.3	41.6	1.00	1.00
32	24	6.71	17.50	13.00	0.91	56.6	42.2	1.03	0.88
33	26	5.80	15.23	13.10	0.92	45.5	39.3	0.96	0.96
34	24	5.71	15.38	13.50	0.94	45.5	39.9	0.98	0.96
35	25	5.24	15.77	15.00	1.05	41.0	39.2	0.96	1.09
36	28	5.45	16.65	15.30	1.07	48.9	44.9	1.09	0.98
37	24	5.57	16.95	15.20	1.06	46.2	41.5	1.01	0.99
38	24	5.75	16.27	14.10	0.99	43.0	37.5	0.92	1.08
39	24	5.52	15.25	13.80	0.97	47.4	43.0	1.04	0.92
40	25	6.12	16.48	13.50	0.94	47.3	38.7	0.95	0.99
41	22	5.86	16.25	13.90	0.97	47.4	40.4	0.99	0.98
42	24	6.21	15.93	12.80	0.90	43.6	35.2	0.85	1.06
43	21	5.30	15.45	14.60	1.02	46.7	44.0	1.06	0.96
44	22	5.97	15.48	13.00	0.91	48.7	40.8	1.00	0.91
45	22	5.55	16.00	14.40	1.00	45.0	40.6	0.99	1.01
46	22	6.33	15.93	12.60	0.88	52.6	41.5	1.01	0.86
47	23	6.23	16.75	13.40	0.94	48.8	39.2	0.96	0.98
48	25	6.41	16.00	12.50	0.87	50.1	39.2	0.96	0.91
49	23	5.94	16.40	13.80	0.97	47.5	40.0	0.98	0.99
50	22	5.74	15.94	13.90	0.97	39.5	34.4	0.84	1.15
51	23	7.35	17.85	12.10	0.85	54.4	37.0	0.91	0.94
52	23	6.57	16.70	12.70	0.89	48.4	36.9	0.90	1.00
53	21	6.19	16.55	13.40	0.94	52.2	42.2	1.02	0.91
54	23	7.53	15.70	10.40	0.73	47.2	31.3	0.76	0.96
55	25	6.64	16.00	12.00	0.84	48.7	36.7	0.90	0.94
56	25	6.88	17.35	12.60	0.88	49.4	35.9	0.88	1.00
57	25	6.61	16.10	12.20	0.85	52.9	40.0	0.98	0.86
58	24	6.35	16.10	12.70	0.89	50.5	39.8	0.97	0.92
59	23	6.50	15.80	12.40	0.87	45.4	35.8	0.88	1.00
60	24	6.46	17.60	13.60	0.95	51.9	40.2	0.98	0.97
61	25	5.09	14.50	14.20	1.00	41.5	40.8	1.00	1.00
62	27	6.29	17.50	13.90	0.97	44.3	35.3	0.87	1.12
63	26	6.24	14.80	11.90	0.83	45.5	36.5	0.90	0.93
64	23	4.82	14.30	14.80	1.04	40.3	41.8	1.01	1.02
65	22	5.35	15.60	14.60	1.02	44.8	41.9	1.02	1.00
66	23	5.87	15.22	13.00	0.91	42.1	35.9	0.89	1.03
67	30	6.24	16.60	13.30	0.93	51.6	41.4	1.01	0.92
68	25	5.58	15.50	13.90	0.97	48.3	43.3	1.04	0.92
69	21	4.94	15.40	15.60	1.09	47.0	47.6	1.16	0.93
70	22	6.18	16.05	13.00	0.91	50.5	40.9	1.00	0.91
71	22	6.34	16.05	12.60	0.89	52.7	41.6	1.00	0.89
72	22	6.13	15.50	12.70	0.89	42.0	34.3	0.84	1.06
73	21	5.55	15.65	14.10	0.99	47.9	43.2	1.04	0.94
74	21	5.44	15.85	14.60	1.02	46.0	42.3	1.03	0.99
75	21	5.86	16.20	13.80	0.97	48.9	41.7	1.01	0.96
76	28	5.65	14.80	12.70	0.89	41.9	37.1	0.91	0.98
77	27	5.86	13.70	11.70	0.82	47.3	40.4	0.99	0.83
78	24	5.38	13.75	12.80	0.90	45.8	42.6	1.03	0.86

TABLE 4.—*Observations on the Blood in One Hundred Normal Young Men—Continued*

Subject	Age	Red Cell Count, Millions per C.Mm.	Hemoglobin, Gm. per 100 Cc.	Hemoglobin per 100 Cc. per 5 Million Cells	Color Index	Volume of Cells per 100 Cc.	Volume of Cells per 100 Cc. per 5 Million	Volume Index	Saturation Index
79	24	6.21	14.30	11.20	0.81	46.0	37.0	0.91	0.90
80	21	5.78	14.35	12.40	0.87	47.3	40.9	1.00	0.87
81	21	6.31	15.65	12.40	0.87	51.0	40.5	0.99	0.88
82	25	5.89	16.20	13.80	0.96	47.4	40.3	0.98	0.96
83	24	6.11	16.20	13.20	0.93	49.4	40.4	0.99	0.94
84	23	6.89	16.42	11.90	0.83	49.2	35.7	0.88	0.96
85	30	5.96	16.28	13.70	0.96	48.7	40.8	1.00	0.97
86	24	6.00	16.38	13.70	0.96	50.3	42.0	1.02	0.94
87	22	6.79	16.75	12.30	0.86	50.2	37.0	0.91	0.96
88	28	5.17	14.20	13.70	0.96	41.5	40.2	0.98	0.98
89	25	5.57	15.85	14.20	0.99	45.8	41.2	1.00	0.99
90	30	5.39	16.42	15.20	1.06	50.3	46.7	1.13	0.94
91	21	5.90	15.18	12.90	0.90	41.1	34.8	0.86	1.05
92	23	6.60	17.00	12.90	0.90	51.0	38.7	0.95	0.95
93	27	5.78	15.80	13.70	0.96	48.7	42.2	1.02	0.93
94	24	5.13	14.30	13.90	0.98	42.6	41.5	1.00	0.98
95	25	5.61	15.30	13.60	0.95	42.9	38.3	0.94	1.01
96	29	6.44	15.24	11.80	0.83	46.2	35.9	0.88	0.96
97	26	5.38	14.65	13.70	0.96	40.9	38.3	0.94	1.02
98	29	5.68	14.45	12.70	0.89	42.9	37.8	0.93	0.96
99	21	5.61	14.36	12.80	0.89	46.9	41.8	1.01	0.88
100	28	5.01	14.30	14.30	1.00	44.3	44.2	1.07	0.91
Average.....		5.85	15.87	13.65	0.96	46.5	39.9	0.97	0.98

SUMMARY

1. The average red cell count in 100 healthy young men between 19 and 30 years of age was 5,850,000, with 95 per cent between 5,000,000 and 6,900,000. In contrast to this, Osgood, in Oregon, found an average of 5,390,000 with 90 per cent between 4,700,000 and 6,100,000.

2. The average total hemoglobin per hundred cubic centimeters of blood in 100 young men was 15.87 Gm., with 87 per cent between 14 and 16.9 Gm. Osgood found an average of 15.76 Gm. with 90 per cent between 14 and 18 Gm.

3. Our average hemoglobin coefficient was 13.66 Gm. per hundred cubic centimeters of blood. Osgood found an average coefficient of 14.66 Gm.

4. Our average color index was 0.95, while the average volume index was 0.97. A slight difference between the color and volume indexes, calculated from data obtained in different parts of the world, is noted.

5. The average total volume of packed red cells in 100 young men was 46.5 cc. per hundred cubic centimeters of blood. Osgood found an average of 46.4 cc. in ninety-four young men.

6. Our average volume coefficient was 39.95 cc. per hundred cubic centimeters of blood. Osgood found an average coefficient of 42.23 cc.

7. It is suggested that the saturation index be defined as "the proportion of hemoglobin per unit volume of cells, expressed in proportion to the normal," and be calculated by dividing per cent of hemoglobin by per cent of volume.

CONCLUSIONS

Accurate blood determinations in the South as compared with similar determinations made in other parts of the world showed a somewhat higher red cell count, with hemoglobin content and volume of packed red cells practically the same as elsewhere. The hemoglobin and volume coefficients were somewhat lower, as were also the color and volume indexes.

The normal blood figures for healthy young men between 19 and 30 years of age, calculated from several hundred accurate determinations made in different parts of the world, are 5,570,000 red cells, 15.88 Gm. of hemoglobin per hundred cubic centimeters of blood and 46.4 cc. of packed red cells per hundred cubic centimeters of blood. The average hemoglobin coefficient of 14.3 Gm. per hundred cubic centimeters of blood should be used as 100 per cent by the various clinical hemoglobinometers, while the average volume coefficient of 41 cc. per hundred cubic centimeters of blood may be taken as 100 per cent in the calculation of volume index.

EXPERIMENTAL BILE PERITONITIS AND ITS TREATMENT IN THE DOG*

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Bile peritonitis has been the subject of much discussion. Some writers have denied that bile plays any rôle in the production of the symptoms that accompany rupture of the gallbladder or bile ducts and have attributed the serious effects to bacterial action.

CLINICAL LITERATURE

Buchanan summarized the literature on bile peritonitis without evident perforation of the biliary tract and came to the conclusion that there were no typical symptoms or signs and that there was insufficient evidence to make such a diagnosis even after operation or post mortem.

Many theories have been advanced as to the cause of this condition: Clairmont and von Hoberer's filtration theory; Newaverch and Luebke's microscopic perforation; Lich and Fraenkel's small almost invisible perforation; a rupture subsequently healed, rupture of the interhepatic bile canal of Newaverch, and postperitoneal perforation of the common duct.

Keene, Da Costa, Judd and Burden, Le Jars, Cumston and Pincoffs and Boggs concluded that bile will do little damage to the peritoneum unless infection is present. Nevertheless, Keene stated that death usually follows within twenty-four hours when there is a traumatic rupture of the gallbladder. Ochsner thought that the serious symptoms are due to fibroplastic peritonitis.

Gosset, Desplas and Bonnet reviewed 111 cases, mostly spontaneous perforation of the gallbladder into the peritoneal cavity, in which operation was performed. The mortality was 52.2 per cent. They stated that perforation of the gallbladder is much more serious than perforation of gastric or duodenal ulcer. The diagnosis is more difficult to make because the clinical symptoms are more rarely met. Meissner reported twelve cases of rupture of the common duct with eight deaths and seven cases of rupture of the hepatic ducts, with five deaths.

EXPERIMENTAL LITERATURE

Clairmont and von Hoberer occluded the common duct experimentally in dogs and reported that four had died with intraperitoneal bile effusion without perforation of the bile tract. Bacteriologic reports were not

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* From the Hull Physiological Laboratories of the University of Chicago.

given. Fraenkel and Kraus used aseptic technic. They did not find any ill effects from opening the gallbladder by laparotomy in guinea-pigs and rabbits, but added that the unsutured wound of the gallbladder cicatrized, and that there were quite a few adhesions about it; they attribute the chief rôle to bacteria.

Whole bile was injected intraperitoneally into rabbits by Bunting and Brown; they found that from 0.25 to 0.5 cc. per kilogram killed the animals in twenty-four hours. Sellards injected autobile and heterobile intraperitoneally into rabbits weighing 1,000 Gm. and found that 15 cc. caused death in a few hours. He also had the same results with guinea-pigs.

Sellards injected beef bile and a bile salt, sodium glycocholate, which had been sterilized in an autoclave, intraperitoneally into rabbits and guinea-pigs, and found that death occurred in from eight to fifteen hours. The bile salt solution used was from 5 to 13 per cent. This strength is excessive, for these salts do not occur in that strength in hepatic bile. Also, the whole bile used was from a different type of animal.

ANALYSIS OF PROBLEM

The questions that naturally arise are in regard to the effect of autogenous bile free in the peritoneal cavity; whether bacteria are the sole harmful agents or whether they do not have any relation to the symptoms and the condition found at autopsy in bile peritonitis; what will be the effect of bile continuously pouring out into the peritoneal cavity; what difference in symptoms will be produced by varying the quantity of bile and what effect will bile from other dogs injected into the peritoneal cavity produce.

METHODS

1. All experiments were performed on dogs anesthetized with ether and operated on aseptically. The abdomen was opened along the edge of the right rectus muscle, the tip of the gallbladder caught up with forceps, a purse-string suture placed about the tip, and then the gallbladder was opened, letting the bile spill out into the peritoneal cavity; the opening was then closed with the purse-string suture, the edges of the bladder being inverted. The intestines were moved about to insure thorough distribution of the bile over the peritoneum, and the abdomen was closed. General symptoms were noted, and various tests applied as indicated.

2. An intraperitoneal fistula of the gallbladder was made aseptically under ether anesthesia. The bile was spilled into the peritoneal cavity and stirred about so that it would come into contact with much of the peritoneum. The edges of the opening in the gallbladder were stitched back, to prevent spontaneous closure, and the abdomen closed.

3. Bile was removed aseptically from the gallbladders of living dogs under anesthesia with a syringe and needle. Bile was collected from eight to twelve dogs, mixed, culture taken, and immediately injected by paracentesis into the peritoneal cavity of dogs, the quantity for single dosage being varied and repeated small doses also being given to other dogs. Part of the bile was sterilized in an autoclave at 15 pounds pressure for thirty minutes before injection.

1. THE EFFECT OF BILE FROM THE GALLBLADDER SPILLED INTO THE PERITONEAL CAVITY

The animal was operated on, February 17. There was slight postoperative vomiting; the animal was fed the second day; food and water were both retained; the animal was rather thirsty; it was up and walking about and ill effects were not apparent; the urine showed a slight trace of bile, and no albumin or sugar; the actions were entirely normal. On May 5, the dog was killed so the pathologic effects of the bile could be noted. At autopsy, the dog was well nourished and in excellent condition. An entirely normal condition was found, except that there were a few adhesions about the tip of the gallbladder where the incision had been made. Cultures were made from the bile at the time of operation and from the peritoneal cavity at autopsy. At operation, staphylococci were found in one case and a bacillus in another. Bacteria were not found at autopsy in any case.

2. THE EFFECT OF BILE CONTINUOUSLY POURING OUT INTO THE PERITONEAL CAVITY

Dog 6.—The animal weighed 10 Kg.; a fistula of the gallbladder was made. The animal vomitted frequently; severe retching occurred; thirty minutes later there was a bowel movement. Later, frequent watery stools were passed; seven hours later, the animal staggered and was much weaker and rather listless; fifteen hours later, the heart was irregular and slow and the respirations slow and sluggish. The dog was hardly able to stand, and gradually became weaker and passed into coma; it did not have convulsions, was difficult to arouse and passed frequent bloody stools; twenty-two hours later, it died in coma; no urine was passed. At autopsy, the lungs were slightly congested in spots; the heart was soft and flabby; the peritoneal cavity contained 275 cc. of slightly bloody fluid deeply stained with bile and a few gelatinous masses. The peritoneum was stained yellow, but this was most marked in the diaphragmatic portion; the peritoneum of the lesser omental cavity was also stained; the liver was markedly congested; bile was not found in the ducts or bladder; the lumen of the gallbladder was open. The intestines were covered with many small petechiae, which were most plentiful over the ileum; the intestines did not contain bile; the mucosa of the colon and small intestines was markedly congested, and the contents of the colon watery and bloody; the greater omentum was stained deep yellow and contained numerous petechiae. The kidneys were congested and bluish-red; the urinary bladder contained 2 cc. of heavy bile colored urine; the chemical tests showed much bile and 5 per cent of albumin. Microscopic examination showed many red blood cells, few white blood cells, also renal cells and débris; cultures were negative.

Dog 4.—A fistula of the gallbladder was made. The animal continued to vomit intermittently for four days, especially soon after eating or drinking. On the fifth day vomiting ceased and recovery was rapid. The urine for the first twenty-four hours contained much albumin and bile pigment which continued heavy for six

days and then gradually diminished, the bile disappearing first. The dog was killed sixty-eight days following operation. At autopsy, the greater omentum was adherent over the liver and the small and large intestines; when it was dissected away, the lobes of the liver were found firmly bound together; when these lobes were separated a sac was opened which contained 10 cc. of bile; this communicated directly through the patent lumen into the gallbladder and ducts; all cultures were negative; the urine obtained at autopsy was normal.

Dog 8.—A fistula of the gallbladder was made. The animal vomited some and had diarrhea for forty-eight hours. On the third day marked edema of the eyelids and lips occurred, as well as anasarca of the subcutaneous tissues of the body; on the third day the edema had diminished somewhat; the urine showed albumin, 5 per cent; many red blood cells; a few white blood cells; much débris and some bile. On the seventh day, the animal was sluggish and moved only when poked. The urine showed albumin and bile; the stool contained bile. The animal died eleven days after operation. At autopsy, the dog was markedly emaciated. There was 50 cc. of bile colored fluid in the peritoneal cavity; partial closure of the lumen in the gallbladder was caused by adhesions of the edges of the liver, leaving only a needle-like opening through which bile passed into the peritoneal cavity; the bile ducts were patent; bile was present in the intestinal contents; the intestinal serosa was congested; the kidneys were congested; the greater omentum was much atrophied, but was adherent to the edge of the liver; cultures were negative.

SUPPLEMENTARY OPERATION

In addition to the operation used in method 2, a supplementary operation was made in which the common duct was doubly ligated and sectioned. This, of course, would cause all the bile to flow from the liver to the gallbladder and out into the peritoneal cavity. The symptoms were similar to those reported for dog 6. Two dogs were used, 14 and 15, each of about the same weight. Both died twenty-three hours after the operation. The conditions found at autopsy were similar. In dog 14, the bacteriologic culture of the peritoneal fluid was negative. In dog 15, *Bacillus coli* was obtained on culture.

Double ligation and section of the common duct only was used in dog 11. The animal progressed favorably as if nothing had been done; on the sixteenth day, it jumped off a table 32 inches high and within a few minutes appeared to be in distress and the abdominal muscles became tense. This was soon followed by vomiting and the other symptoms of bile peritonitis. Death occurred twenty-four hours later. The condition found at autopsy was typical of bile peritonitis; a small perforation of the common duct 1 inch (2.5 cm.) above the ligature permitted bile to flow freely into the peritoneal cavity when the gallbladder or hepatic ducts were compressed. Diagnosis in this case was indirect traumatic rupture of the distended common duct followed by bile peritonitis, which is a condition similar to obstruction of the common duct by stone or tumor, with distention of the gallbladder and ducts in man, with rupture from slight trauma followed by peritonitis.

A few days later Dr. Ivy observed a similar acute onset of symptoms in a dog with which he was working. The dog died within twenty-four hours, and at autopsy showed a ruptured bile duct.

In section 2 it has been shown that bile is toxic and will produce death. The following technic was devised to determine the quantity of bile necessary to kill when free in the peritoneal cavity. As the dogs varied so much in weight, all injections were made on the basis of weight, so many cubic centimeters per kilogram of body weight.

3. THE EFFECT OF INTRAPERITONEAL INJECTION OF BILE

Dog 13.—Into an animal weighing 4.2 Kg., 25.2 cc. of bile from the gallbladder was injected intraperitoneally, 6 cc. per kilogram of body weight. The symptoms were similar to those in the foregoing cases, but progressed more rapidly; death occurred in fifteen hours. The conditions found at autopsy were similar except that there was more clotted blood in the peritoneal cavity. Cultures were negative.

Dog 17.—An animal weighing 4 Kg. was given 20 cc. of bile from the gallbladder intraperitoneally, 5 cc. per kilogram of body weight. The symptoms were similar to those in the foregoing cases; death occurred ten hours after the injection. The condition found at autopsy also was similar to that found in the foregoing cases. Culture showed *B. coli* contamination.

Dog 33.—Into an animal weighing 18 Kg., 90 cc. of bile from the gallbladder was injected intraperitoneally, 5 cc. per kilogram of body weight. The usual symptoms and condition were found at autopsy. The animal died after eleven and a half hours. Cultures were negative.

Dog 34.—Into an animal weighing 12 Kg., 60 cc. of bile was injected intraperitoneally, 5 cc. per kilogram of body weight. The usual symptoms and condition were found at autopsy. The dog died after eleven and a half hours. Cultures were negative.

EFFECT OF SUBLETHAL DOSE GIVEN INTRAPERITONEALLY

Dog 23.—Into an animal weighing 4.2 Kg., 16.8 cc. of bile from gallbladder was injected intraperitoneally, 4 cc. per kilogram of body weight. There was immediate spasticity of the abdominal muscles, and the dog appeared to be in distress. (Intraperitoneal injections of physiologic sodium chloride solutions did not cause rigidity and only slight pain when paracentesis was performed.) Five minutes later, defecation and retching occurred. Ten minutes later vomiting and staggering were noticed; the retching and vomiting were repeated every few minutes—this was accompanied by thirst and lapping of water and frequent stools which became watery and then bloody. Nine hours later, there was marked edema of the eyelids and lips; a small amount of urine was passed, which contained bile and a trace of albumin; the heart was slow and irregular; respirations were slow. Eighteen hours later, the condition had returned practically to normal except for anuria. Thirty hours later, the urine showed bile and much albumin; some edema of the eyelids occurred. On the seventh day, the condition was good except for slight fluctuating edema of the eyelids; the urine showed bile and albumin. On the twenty-eighth day, edema of the eyelids persisted; the urine showed albumin in varying amounts. On the thirty-first day, the animal died. Autopsy showed many patches of bronchopneumonia in the lungs; the heart was flabby; 50 cc. of bile colored peritoneal fluid was obtained; the liver showed a nutmeg appearance; the kidneys were congested; the suprarenals were diminished, especially the medulla.

Dog 42.—Into an animal weighing 3 Kg., 6 cc. of bile from the gallbladder was injected intraperitoneally, 2 cc. per kilogram of body weight. The symptoms were the same as in the foregoing cases, but were less severe. Twenty-four hours later, the condition was normal, except the urine contained bile and albumin; the albumin gradually diminished; none was found on the seventh day. The dog was normal two months after injection.

INFLUENCE OF BACTERIA

In a few instances, bacterial growths were obtained from the bile of other dogs used for these injections. Bacteria were also obtained from the peritoneal fluid following death of the dog into which bile had been injected; the cultures contained staphylococci or colon bacilli. The bacteria in most instances certainly could not cause the symptoms and death as the same reactions were obtained in dogs in which bacteria were not found in the bile used or in the peritoneal fluid at postmortem. Nevertheless, for evidence on this point, the following experiment was done:

Dog 30.—Into an animal weighing 1.6 Kg., 8 cc. of sterilized bile was injected intraperitoneally, 5 cc. per kilogram of body weight. Death occurred in twenty-two hours. The symptoms and the condition found at autopsy were the ones usually seen. Cultures were negative.

SUMMARY

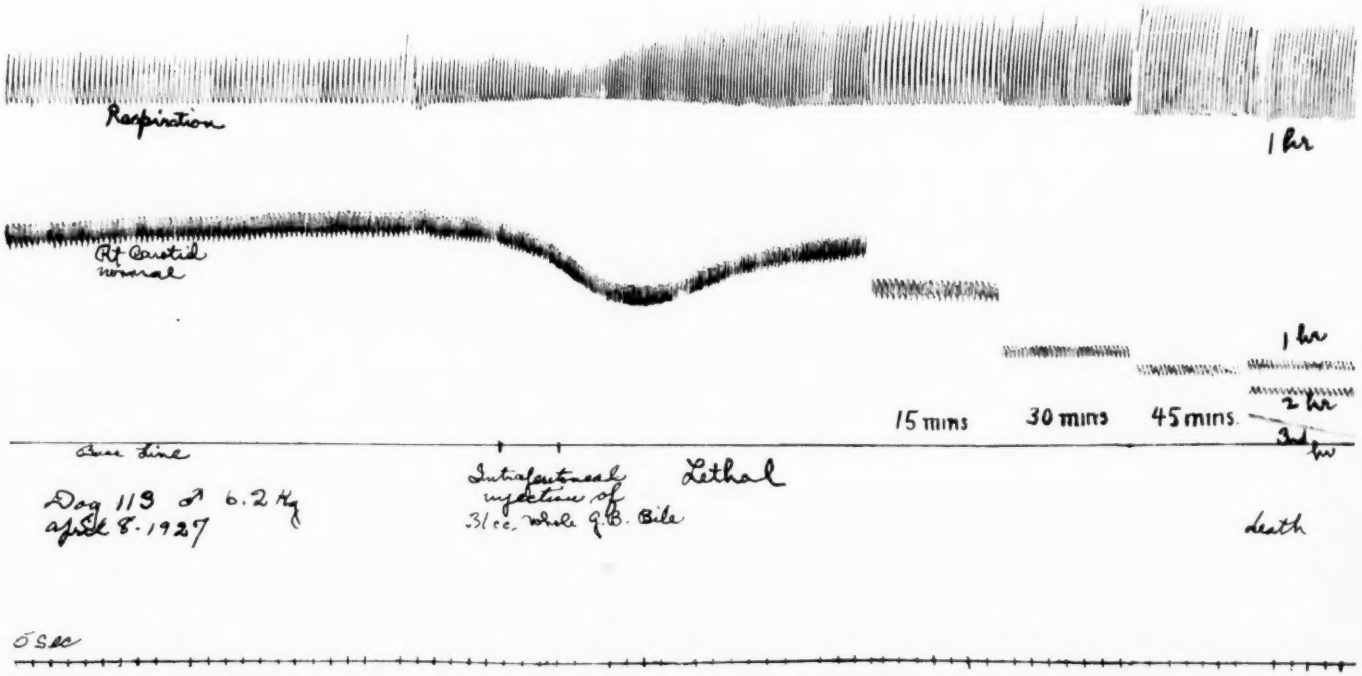
Bile from the gallbladder was spilled into the peritoneal cavity in twenty-five dogs. There were no deaths, and the effects were slight and transitory. Some of the dogs vomited, a few had diarrhea, and the urine contained varying amounts of bile pigment. The amount of jaundice present was just perceptible. Bacteria were not the cause of these symptoms, as sterile or infected bile gave the same results.

Intraperitoneal fistula of the gallbladder in seven dogs permitted sufficient bile to pass into the peritoneal cavity to cause death within twenty-four hours. If the fistula was partly closed, the dog lived longer, and if it was completely closed early, the effects were transitory. The symptoms preceding death were: bradycardia, low blood pressure, slow respiration, anuria and coma, and in many cases edema.

Intraperitoneal injection of bile from the gallbladder of dogs into nine dogs in amounts of 5 cc. or more per kilogram of body weight caused death within twenty-four hours. Sterilized bile had the same effect as shown in the accompanying illustration.

GENERAL COMMENT

The first series of experiments questioned the supposed relation of bile to peritonitis, and brought forward the question as to the combined action of bacteria and bile in the production of the peritonitis. Various authors have stated that bile was practically harmless when sterile, and attributed most of the serious cases to the infected contents of the gallbladder. One writer stated that almost all gallbladders containing stones are harboring bacteria which become virulent when free in the peritoneal cavity. But another writer stated that the bile is generally held to be antiseptic and antitoxic. If the latter were true there would be little need for the present elaborate care in the technic of operations on the gallbladder, and a perforation of the gallbladder or ducts should not cause worry.



Record of dog given ether-barbital anesthesia. Intraperitoneal injection of 31 cc. of whole bile from the gallbladder, showing gradual failure of the heart and the blood pressure. Death at the end of three hours.

But when a permanent fistula was made between the gallbladder and the peritoneum, all the dogs died within twenty-four hours. Bacteria were found in only a few cases, and apparently had little if any effect on the course of the disease. The only conclusion that could be drawn was that more bile entered the peritoneal cavity by way of the fistula than when the gallbladder was merely opened, emptied and closed. In addition, ligation of the common duct precluded the possibility of bile passing into the intestines, and all these dogs died within twenty-four hours. It appears that when there is a lumen in the gallbladder wall, practically all the bile passes out into the peritoneal cavity, as there is not enough pressure in the ducts to cause passage of bile into the intestines along the normal pathway. The ligature also prevented the possibility of pancreatic juice passing out into the peritoneal cavity by way of the gallbladder and adding a complicating factor.

The whole question resolved itself into one of the quantity of bile in the gallbladder. Hence, bile was taken from the gallbladders of sixty-two dogs and measured; the largest amount obtained from one dog was 24 cc.; this dog weighed 19 Kg. The smallest amount was 3 cc.; the average was 8.1 cc., and the average weight of the dogs 9 Kg. Accordingly, when the gallbladder was merely opened, emptied and closed, an average of only 0.9 cc. per kilogram of body weight got into the peritoneal cavity. When the lumen is left open, certainly a much larger quantity enters within a few hours. Bile from the gallbladder was then injected intraperitoneally in varying amounts up to 6 cc. per kilogram of body weight and it was found that 5 cc. per kilogram of body weight caused death within twenty-four hours. Sterilized bile had the same effect as unautoclaved bile.

Clinical Symptoms and Signs of Bile Peritonitis in Dogs.—Rigidity of the abdominal muscles, especially in the right upper quadrant, and retching and vomiting occur; pain may be extremely severe; early defecation and later, watery or bloody stools occur; bradycardia, irregular heart, weak pulse, slow and irregular respiration, early restlessness, later, lethargy, and finally coma and death occur; urine, if obtainable, contains bile and albumin (usually total anuria); edema was present in 30 per cent of the dogs; jaundice was slight and sometimes questionable. Apparently the renal portal in dogs is low for bile pigments, as they are rapidly excreted in the urine.

TREATMENT OF BILE PERITONITIS

An attempt was made to treat animals with bile peritonitis by intravenous injections, with the idea that it might be possible to neutralize the action of bile salts as they came into the blood stream and thereby prevent them from injuring the heart, kidneys or other vital organs, and

to sustain the blood pressure and heart action. Epinephrine was also used (dogs 111 and 114) after a lethal dose of bile had been given, and it was found that epinephrine had only a temporary effect (from three to eight minutes); that the course of the disease was the same after the effect of the epinephrine, which was transitory, had passed and that the course was unmodified, death occurring at the same time as if epinephrine had not been used. It was hoped by this method to raise the blood pressure to such an extent that the kidneys would continue to excrete, thereby eliminating the toxic bile salts from the circulation. Ephedrine had a slightly prolonging effect, but the course of the disease was not modified.

The dilution of the bile and bile salts when injected intraperitoneally and also their dilution when injected intravenously were considered, so that a fairly weak solution would be injected, which would not take effect so rapidly. The use of sodium chloride with bile and bile salts in the intravenous injections appeared to give a slower rate of action, but if the total quantity of bile was injected within one hour, the toxic effect was the same as with the lesser dilute solutions.

The rate of injection markedly influences the toxic action of bile, as was demonstrated by Meltzer and Salant in 1906, but when a given amount of bile salts or of bile is injected within a period of one hour, even though it may be injected slowly, the apparent total effect is the same. Of course, if only a small quantity of bile is injected, less than a lethal dose per kilogram of body weight, for example, 2 cc., and injected as rapidly as it can be put into the blood stream, there may be immediate death. This occurred in dog 22, when whole bile was injected intravenously. The dog died immediately. Autopsy showed an immense thrombus extending even through the heart and into the lungs. This, of course, would mean an immediate massive destruction of blood cells, and the effect is not due to the toxicity of the bile so much as it is due to the mechanical effect of the thrombus. Accordingly, after that all injections were made slowly enough to avoid the formation of thrombi. When bile is injected slowly, a formation of thrombus or a fatality from embolism rarely occurs. Bile, of course, lakes the blood but I have never had more than a 10 per cent laking of the blood, and this is not sufficient to give reaction from that alone. Serum containing 10 per cent hemoglobin from blood taken after intravenous injection of bile (dogs 123 and 118) was injected intravenously into dogs 125 and 119, respectively. It did not cause a reaction. Accordingly, I cannot agree with Meltzer and Salant that large quantities of bile can be injected without toxic effects if one places the limit of the injection of the bile or bile salts at the lethal dose within one hour, and that has been used as the standard.

Chemical Methods of Abating Bile Peritonitis.—The literature contains frequent references to the effects obtained by injecting calcium, on the theory that calcium combines with bilirubin, making a hypocalcemia or diminishing the available calcium. A lethal dose of whole bile was injected intraperitoneally into dogs and calcium chloride, 10 per cent, given intravenously at various intervals, but the dogs died, as usual, in twenty-four hours. Because of the transitory nature of excess calcium in the blood and the exceedingly rapid action of bile in the blood, various intervals of time were used between the injection of calcium and bile in the blood; also, various quantities of calcium chloride were used, but the course of the disease was not affected. Calcium chloride was then mixed with whole bile before a lethal dose of bile was injected into the peritoneal cavity, and to my surprise the dogs lived only half as long as the average dog lived without the use of calcium chloride. Apparently, then, the action of calcium chloride was the exact reverse of what was expected. The experiment was repeated several times, and the results were the same in all cases, that is, there was not only no protective action, but the length of life was shortened by a half. The question then came up as to the toxicity of the calcium chloride which was injected. For a control, the same quantity of the same preparation of calcium chloride was injected into the peritoneal cavity, and toxic effects did not occur (table 1).

Calcium Lactate.—Stewart and King stated that they rendered the bilirubin inactive by the use of calcium lactate. Calcium lactate was mixed with whole bile and the mixture injected intraperitoneally, and the dog lived for ninety-six hours. At autopsy, the abdomen was literally filled with adhesions and exudate, the most extensive that had yet been observed. To be sure, life was prolonged, but the marked tendency to adhesions would certainly be serious. The second dog received calcium lactate and bile intraperitoneally and died in twelve hours. Autopsy showed a large quantity of fluid in the peritoneal cavity—typical bile peritonitis. The control dog in which calcium lactate alone was injected lived for several weeks.

Cholesterol and Lecithin.—Cholesterol and lecithin were considered because of their association with bile in the gallbladder. Pure cholesterol and lecithin were each mixed separately with whole bile and injected intravenously. The toxic effects were slightly diminished. When they were injected intraperitoneally, a modification in effects was not noticed. So apparently, then, the cholesterol and lecithin when injected with pure bile salts or with the bile itself modify the toxic effect only slightly.

Fats.—In a recent article by Potter on the treatment of fistula following operation on the gallbladder, drains soaked in olive oil were advised. I had previously thought that perhaps the bile in the intestine is

physiologically rendered inactive by its early combination with fats. Accordingly, I used olive oil mixed with bile and bile salts for intraperitoneal injection, and in all cases found that the dogs died much sooner than they would have died without the use of olive oil. The

TABLE 1.—*Intraperitoneal Injection of Whole Bile from the Gallbladder and Bile Substances* *

Dog	Weight, Kg.	Material	Amount, Cc.		Results
126	5.0	Bile	25	8 a.m., calcium chloride, 10%, 5 cc., intravenously 3 p.m., calcium chloride, 10%, 5 cc., intravenously 8 p.m., calcium chloride, 10%, 5 cc., intravenously	Dead after 24 hours; typical bile peritonitis
127	2.0	Bile	10	Calcium chloride, 9 cc., intraperitoneally	Dead after 11 hours; intestinal contents bloody
128	6.0	Bile	30	Olive oil, 20 cc., intraperitoneally	Dead after 5 hours; bile peritonitis
129	3.7	Bile	18.5	Calcium lactate, 5%, 10 cc., intraperitoneally	Dead after 96 hours; many peritoneal adhesions; bile peritonitis
130	9.5	Bile	47	9 a.m., physiologic sodium chloride, 200 cc., intraperitoneally 3 p.m., physiologic sodium chloride, 200 cc., intraperitoneally 8 p.m., physiologic sodium chloride, 200 cc., intraperitoneally	Condition remained good; living after 35 days
132	2.2	Bile	11	Olive oil, 4 cc., intraperitoneally	Dead after 7 hours; peritoneum pale
133	4.0	Bile	20	Calcium chloride, 10%, 10 cc., intraperitoneally	Dead after 6 hours; peritoneum hyperemic; fluid
134	4.0	Bile	20	Calcium lactate, 5%, 20 cc., intraperitoneally	Dead after 12 hours; fluid in peritoneal cavity
135	5.2	Bile	31	4 p.m., sodium chloride, 0.8%, 100 cc., intravenously 11 p.m., sodium chloride, 0.8%, 100 cc., intravenously 8 a.m., sodium chloride, 0.8%, 100 cc., intravenously 2 p.m., sodium chloride, 0.8%, 50 cc., intravenously	Dead after 5 days; adhesions throughout abdomen; fluid in abdomen, 200 cc.
136	2.8	Bile	14	4:30 p.m., sodium chloride, 0.8%, 100 cc., intraperitoneally 11 p.m., sodium chloride, 0.8%, 100 cc., intraperitoneally 8 a.m., sodium chloride, 0.8%, 100 cc., intraperitoneally 3 p.m. sodium chloride, 0.8%, 50 cc., intraperitoneally	Dead after 6 days; no adhesions in abdomen; cause of death not determined
137	5.0	....	..	Calcium lactate, 5%, 20 cc., intraperitoneally	Dead after 8 days; pneumonia
138	5.2	....	..	Calcium chloride, 10%, 10 cc., intraperitoneally	Living after 20 days; well
139	7.0	....	..	Olive oil, 20 cc., intraperitoneally	Living after 20 days; well

* Bile was mixed with various chemicals to test the inhibitory effects of such chemicals on the toxicity. Calcium chloride, calcium lactate and olive oil did not have an inhibitory power but even in most instances caused acceleration of the poisonous action of bile. Injections of these chemicals alone into control dogs did not cause toxic symptoms. Large quantities of sodium chloride solution given intravenously and especially intraperitoneally produced the most favorable results.

control dog in which olive oil alone was injected intraperitoneally was living twenty days later and was normal. The results suggested the theory that the fats rendered the bile salts much more rapidly absorbable, and instead of the immense amount of fluid being poured out into the

abdomen and the bile salts being absorbed at such a rate that they caused death within twenty-four hours, the bile salts with fat were absorbed so that death occurred within from five to seven hours.

Surgical Treatment of Bile Peritonitis.—In a number of dogs the gallbladder was opened and left open as in the early operations, and after varying intervals of time, the abdomen was aseptically reopened and the gallbladder closed. Various intervals of time were set for the closing of the gallbladder, all of which must be within twenty-four hours. At the time the gallbladder was closed, the peritoneal cavity was thoroughly washed out with sodium chloride solution and a quantity of the solution was left in the peritoneal cavity. The dogs in which the second operation was done at eighteen hours all died; one lived at sixteen hours; all lived at twelve hours or less. Apparently, then, the maximum time at which the operation can be done following rupture of the gallbladder is sixteen hours; the preferable time is twelve hours or less. This, of course,

TABLE 2.—*Typical Results of Operative Treatment of Bile Peritonitis* *

Dog	Operation	Treatment	Result
82	Laparotomy: gallbladder was opened and left open; abdomen closed as in usual operation; aseptic technic	8 hours later the abdomen was reopened and the lumen in gallbladder closed; peritoneal cavity thoroughly washed out with 1,000 cc. of sodium chloride, 0.8%, and 200 cc. left in abdomen and the abdomen closed; aseptic technic	Lived
83	Same.....	12 hours later, the same procedure was followed as in dog 82	Lived
85	Same.....	18 hours later, the same procedure was followed as in dog 82	Died

* Dogs that had a ruptured gallbladder with bile pouring out into the peritoneal cavity lived if they were operated on early, that is, within sixteen hours following the rupture, and the peritoneal cavity washed out with salt solution.

presupposes the supposition that enough bile has entered the peritoneal cavity so that death will occur in twenty-four hours. In addition to the closing of the gallbladder and the washing of the peritoneal cavity, an intravenous injection of sodium chloride solution causes the animal to return to normal condition more rapidly, raising the blood pressure and increasing the activity of the kidneys. Water by mouth could not be used in these animals because of the persistent vomiting, neither can fluid be used by rectum because of the frequent diarrhea. The dogs always drink excessively when water is before them, but almost immediately vomit the entire contents of the stomach, so that little can be gained by attempting the intestinal route. Intravenous injection is preferable to hypodermoclysis (table 2).

CLINICAL APPLICATION

From a review of the literature in which the diagnosis of bile peritonitis has been made at autopsy or at operation, it seems that rupture of the gallbladder or biliary ducts is extremely serious—a mortality rate of

50 per cent or more. The cause of the high mortality would appear to be due in large part to the failure to make an early diagnosis and to recognize the seriousness of the condition. Such patients are usually seen by the family physician and the industrial surgeon. A delay of a few hours will cost the life of the patient.

The outstanding symptoms in experimental bile peritonitis are: sudden spasticity of the abdominal muscles; nausea and vomiting; continuous pain, generalized in the abdomen; restlessness; early defecation, then diarrhea with bloody stools, and bilirubin in the urine. Later, the heart becomes slow and irregular; blood pressure falls; the respiration is slow or of the Cheyne-Stokes type; albumin and blood are found in the urine and coma occurs. When these late symptoms appear, the animal is in a precarious condition.

Chemical treatment, such as calcium chloride given intravenously and intraperitoneally, was of little if any value. Epinephrine and ephedrine sustained the heart temporarily, but this effect was transient. Salt solution given intravenously and subcutaneously did prolong life. Experimentally, it was found necessary to operate within the first sixteen hours, as the dogs did not live when operated on later than that. Practically all the dogs were saved when operation was performed prior to twelve hours after the rupture. The use of oils, calcium salts and cholesterol and lecithin in the peritoneal cavity was without value, and in some instances hastened the poisonous action of bile.

The treatment found to be the best was: closing the opening in the gallbladder or bile passages; thorough lavage of the peritoneal cavity with warm physiologic sodium chloride solution, and then leaving a few hundred cubic centimeters of the salt solution in the abdomen followed by complete closure. With this, intravenous injection of salt solution was of value.

It is extremely important that a correct diagnosis be made within the first few hours following spontaneous or traumatic rupture of the biliary system, and that proper surgical treatment be instituted.

CONCLUSIONS

Bile from the gallbladder in the peritoneal cavity of dogs in amounts of 5 cc. or more per kilogram of body weight causes peritonitis and death within twenty-four hours.

Bacteria are not essential and do not modify the course of bile peritonitis when bile is present in a sufficient quantity to cause death within twenty-four hours.

From this series of experiments, it seems that bile peritonitis is a clinical entity.

Calcium chloride and calcium lactate neither inhibit nor diminish the toxic action of bile.

Olive oil accelerates the toxic action of bile.

Cholesterol and lecithin slightly inhibit the toxicity of bile.

Epinephrine and ephedrine have favorable effects, but the effects are exceedingly transient.

Physiologic sodium chloride solution given intraperitoneally, intravenously or subcutaneously has the most favorable effect on the course of the disease.

Early diagnosis and immediate operation are imperative.

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HYPERBILIRUBINEMIA IN PEPTIC ULCER*

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Clinical jaundice is sometimes an accompaniment of duodenal ulcer when no other reason than the ulcer is apparent for its existence. Thus, it has been described as a symptom of duodenal ulcer by Reed,[1] Ewald,[2] Kemp,[3] Schmidt [4] and others. Usually it is described as a rare symptom [5] but Collin [6] found jaundice in nine of sixty-two cases of duodenal ulcer.

With the development of more exact methods of estimation of bilirubin in the blood, the question of possible jaundice in duodenal ulcer has become interesting. For, if clinical jaundice is present either as a rare symptom of ulcer or occurs in some series oftener than once in ten cases and, since the location of duodenal ulcer is fairly constant, it would seem possible that a certain proportion, possibly a large group, of patients with duodenal ulcer would show latent jaundice. If this is true, the interpretation of hyperbilirubinemia might be confusing unless duodenal ulcer had been carefully excluded. It has even been suggested [7] that duodenal and gastric ulcers might be differentiated by means of calculation of the icterus index.

Different and confusing reports of the bilirubin content of blood in patients with duodenal ulcer have been made. Bernheim [7] found the icterus index above normal in sixteen patients with duodenal ulcer, while twelve patients with gastric ulcer showed normal figures. In a series of thirty persons with ulcers, Scherck [8] found thirteen with distinct elevation of bilirubin; he used van den Bergh's method, measuring in bilirubin

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* From the Third Medical Division of Bellevue Hospital.

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units and taking 1 as a high normal limit. He found the blood bilirubin above normal in seven patients with duodenal, in three with pyloric and in three with gastric ulcers. The highest reading of the duodenal series was 3 and the average of the abnormal readings, 1.84—. Of the six persons with pyloric ulcers, three showed a bilirubin content above normal, with an average of 1.28 for the three. Of ten patients with gastric ulcers, the bilirubin in three was above 1, with an average of 1.25—. Speik, Liljedahl and Falk [9] reported that hyperbilirubinemia was present in sixteen patients with duodenal and gastric ulcer. They used the Fouchet test. They stated that there was no evidence of disease of the gallbladder in these cases, but did not state what means were used to exclude such possibilities. This test was positive in one patient with bronchial asthma, two with angina pectoris, four with pulmonary tuberculosis and others. Haak [10] thought that some persons with duodenal ulcers give high blood bilirubin calculations. He saw five such cases. On the other hand Friedman and Strauss [11] found that four patients with gastric and six with duodenal ulcers did not show hyperbilirubinemia. They concluded that the presence of hyperbilirubinemia is of value in deciding between cholecystitis and gastric and duodenal ulcer or carcinoma. Arnoldi and Schechter [12] estimated the bilirubin of the blood in persons with secretory disturbances of the stomach. They found it low normal in those with hyperacidity and high normal in those with lowered acidity and achlorhydria.

It has been suggested by Barrow, Armstrong and Olds'[13] that adhesions about the duodenum block the ducts and cause the jaundice. Other explanations have included swelling [8] near the papilla of Vater with mechanical obstruction and a vagotonic state [8] resulting from the ulcer, with reflex spasm of the ducts.

In the papers already mentioned, when hyperbilirubinemia was found in patients with duodenal or gastric ulcer, mention was not made of the steps taken to exclude other abdominal disease. Thinking that this might be a source of disagreement, we have selected from cases occurring in the wards of the Third Medical Division of Bellevue Hospital and the Clinic of Gastro-Enterology of New York University ten

9. Speik, F. A.; Liljedahl, E. N., and Falk, M. A.: Observations on the Fouchet Test in Latent Jaundice, J. A. M. A. **82**:2092 (June 28) 1924.

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patients with gastric and thirteen with duodenal ulcer, in whom roentgen-ray study, physical examination and history exclude disease of the gallbladder and gall duct and periduodenal adhesions as far as possible. Blood serum was collected from these twenty-three patients and the direct van den Bergh and the quantitative indirect tests and determinations of the icterus index were made.

The technic for the icterus index was that of Meulengracht, as modified by Gram and by Bernhard and Mane and as given by Bernheim.[7] The method for the van den Bergh reaction as given by McNee [14] was followed carefully.

The results of this study are given in the accompanying table.

Results of Study of Twenty-Three Patients with Ulcers

Location of Ulcer	Direct	Indirect Units	Color Index
Stomach	Negative	0.1	.
Stomach	Negative	0.5	4
Duodenum	Negative	0.6	5
Duodenum	Negative	0.5	4
Duodenum	Negative	0.6	5
Duodenum	Negative	0.4	3
Duodenum	Negative	0.9	6
Stomach	Negative	0.4	3
Stomach	Negative	0.4	3
Stomach	Negative	0.4	3
Duodenum	Negative	0.3	3
Pylorus	Negative	0.5	4
Duodenum	Negative	0.4	3
Duodenum	Negative	0.4	3
Pylorus	Negative	0.4	3
Duodenum	Negative	0.3	2
Stomach	Negative	0.75	5
Stomach	Negative	0.5	4
Duodenum	Negative	0.3	2
Duodenum	Negative	0.3	2
Duodenum	Negative	0.6	4
Duodenum	Negative	0.4	3
Stomach	Negative	0.5	4

SUMMARY AND CONCLUSIONS

Conflicting reports as to the occurrence of latent and actual jaundice in persons with duodenal ulcer led us to investigate twenty-three cases of duodenal and gastric ulcer in which roentgen-ray and other examination did not reveal, evidence of a pathologic condition of the gallbladder or gall duct or adhesions in the upper part of the abdomen. Determinations of bilirubin in the blood serum of these patients showed a normal amount in every instance. This confirms the observations, in the smaller series, of Friedman and Strauss. These results would tend to support the suggestion that jaundice when present in persons with duodenal ulcer is either caused by periduodenal adhesions, catarrhal inflammation of the duodenum or a complication in the biliary system.

14. McNee, J. W.: Jaundice: Review of Recent Work, Quart. J. Med. **16**: 390 (July) 1923.

CLINICAL SPIROGRAPHY

SPIROGRAMS AND THEIR SIGNIFICANCE*

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Since 1923, all determinations of the basal metabolic rate conducted in the Battle Creek Sanitarium Clinic have been made by the graphic method. These graphs form the subject of our present study. We have chosen to call them spirograms to differentiate them from the numerous other types of graphs used in medicine.

While a casual observation of these tracings gives the impression that every conceivable irregularity may be obtained, a closer inspection reveals that there is a certain order in the apparent chaos. One of us (P. R.) first noted that the spirograms could be divided into about ten types, each with definite characteristics. For the past four years all spirograms have thus been read and classified on a purely graphic basis by one technician. We take pains to state that the reader of the spirograms had not the slightest idea whether they had any clinical significance, but was interested only in accurate observation of the tracings. Prejudice or bias based on unconscious clinical observation, therefore, cannot play a rôle in the classification.

When about 20,000 tests had been made, we undertook to determine what the significance of these spirograms might be. Unexpected and valuable data have been discovered. The diagrams with original instructions as given to the technician are reproduced in figure 1.

A normal pneumogram has been described repeatedly and is recognized in standard physiology. A spirogram obtained by the usual graphic metabolism apparatus is practically identical with this. The pen ascends with each inspiration. The depth of inspiration varies considerably more than the depth of expiration, because expiration is a passive act. A line drawn, or imagined drawn, to connect the inspiration apexes is called the inspiratory line. This is the upper margin of the tracing. A similar lower margin gives the expiratory line. (The geometrical line drawn close to the lower margin is entered by a technician for purposes of calculating the basal metabolic rate and has

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nothing to do with typing the tracing.) A normal tracing has a regular and even expiratory line and a slightly irregular inspiratory line. There is a slight pause at the end of each expiration.

Type *A* is almost identical with a normal respiration. It is characterized by a fairly regular inspiratory (upper) line and a regular expiratory (lower) line. In addition, there is a slight pause at the end of each expiration. There are two subtypes (*A* and A_2), the second showing more irregularity than the first.

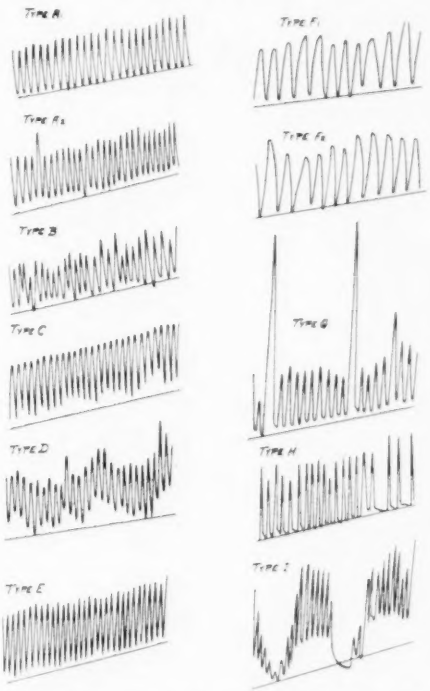

Fig. 1.—Diagrammatic illustration of the nine respiratory types here presented. In type *H* the tracing is not long enough to show the waviness referred to in the description. See figure 3.

Type *B* is determined by an irregular irregularity of the inspiratory and expiratory lines, especially the former; otherwise it is like the irregular type *A* (A_2).

Type *C* is an inverted type. The inspiratory line is practically straight, while the expiratory line is somewhat irregular. The slight pause in each respiration occurs at the end of inspiration instead of at expiration.

Type *D* shows a periodic and fairly regular wavy contour in both inspiratory and expiratory lines—a ribbon-like formation. The regularity distinguishes it from type *B*.

Type *E* presents a normal general contour, but in the individual respirations there is no pause either at inspiration or at expiration.

Type *F* is characterized by a normal general contour, but there is a definite pause at inspiration. In this sense it is a reversal of the normal.

Type *G* has the normal inspiratory line interrupted by frequent and extremely deep inspirations. The intermediate portions of the tracing are normal.

Type *H* shows a definite regular waviness in the inspiratory line. In addition, there is a pronounced pause at each expiration.

Type *I* is the well known Cheyne-Stokes type. There is a periodicity in the entire contour, a tendency to crescendo and diminuendo in the individual respirations and a pause at expiration considerably greater than that encountered in type *H*.

It is not feasible in this brief paper to give the data or all of the reasoning that led up to our conclusions. This will have to appear in detail elsewhere. We shall merely state briefly the general method of procedure.

Two approaches were used in order to avoid the errors likely to creep into a statistical study. The first was the approach from types to clinical observations, the second from clinical observations to types. In other words we selected first all the cases of one type and determined their characteristics; then we selected all cases of one characteristic and determined in which types they occurred. The cases were divided into constant and inconstant, according to whether the same type of respiration was manifested in every test or whether different types occurred in the various tests.

CHARACTERISTICS OF THE TYPES STUDIED

To determine the characteristics of the types, only the constant cases were used. These were studied from the standpoints of age; race; sex; state of nutrition; basal metabolic rate; pulse rate; respiration rate; results of urinalyses, acidity, sugar, albumin, acetone, casts and blood cells; observations on the blood cell count, hemoglobin content by the Dare method, Wassermann test, nonprotein nitrogen, uric acid and sugar content; alveolar carbon dioxide tension and acetone; ventilation of the lung in liters per minute, and all diagnoses. The incidence of every item was first determined on the normal series, and this was used as a control. Unless a condition occurred in any type to such a degree that

it differed from the normal by one-third, it was not admitted as an established abnormality.

A study of the inconstant cases was productive. There were two types of inconstancy, one in which a tracing partook simultaneously of the characteristics of two typical spirograms and cases which fell in one type in one test and in other types subsequently. This gave us a good insight into the type interrelationships. We found that there was a definite association between certain types and apparently an impossibility of association between others. Only twelve of thirty-six theoretical possibilities actually occurred (fig. 2). The most striking feature of figure 2 is the fact that it shows that a patient with a normal

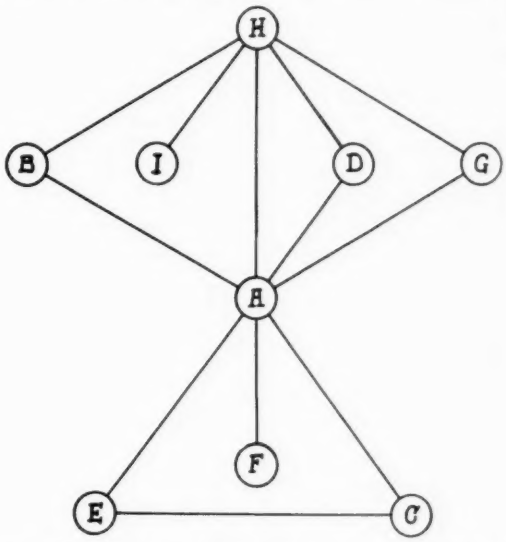

Fig. 2.—Diagram to show type interrelationships. The central situation of type *A* and its connections with all types except *I* can be noted. Types *F* and *I* each have only one relationship.

respiration cannot possibly develop a Cheyne-Stokes type (*I*) unless he first develops type *H*.

The clinical characteristics of the various types as determined by this work are as follows:

Type *A* is the normal and serves as the control. It may be the precursor of others when sufficient cause arises to alter the type. It is related to all other types except *I*. There is hardly a disease process incompatible with a normal type of respiration, but other factors, e.g., puberty or chemical changes of the blood, may incur to bring about a change.

Type *B* may arise by a dynamic process in the endocrine system. Its most common associations are with types *A* and *H*. Conditions which occur in this type considerably oftener than in the normal type are: hypotension, neurotic and psychotic states, mucous colitis and endocrine disturbances. Type *C* occurs practically exclusively in women, and hence we believe that it is controlled by some unusual function of the female hormone. We are not in a position to make any other statement concerning it, because of the paucity of our material.

Type *D* is largely, though not exclusively, masculine (about 88 per cent). It occurs in young boys as early as they can be tested (before puberty). In this type there is a peculiar mixture, a tendency to endocrine disturbances with both hyperthyroidism and hypothyroidism, and also a tendency to cardiovascular-renal disease. This is manifested by

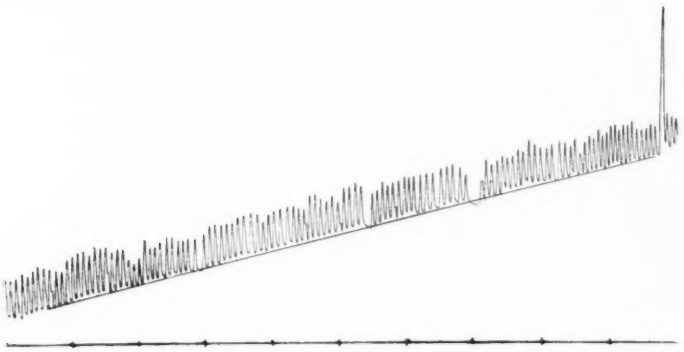

Fig. 3.—Spirogram illustrating the wavy appearance of the inspiratory line and the exaggerated pause at the end of expiration, which are the characteristics of type *H*.

the retention in the blood of nonprotein nitrogen, uric acid and sugar. Osteo-arthritis and obesity also are prevalent in this type, while neurotic and psychotic manifestations and constipation are rare. The associations are with types *A* and *H*.

Type *E* though graphically appearing entirely different from the former is nevertheless somewhat similar clinically. It resembles type *D* in retention of nonprotein nitrogen, uric acid and sugar in the blood and in the occurrence of osteo-arthritis. But it differs from *D* in prevalence of colitis and neurotic states and in the absence of endocrine disturbances. Its associations are with types *A* and *C*.

Type *F* is one of the rare types and has only one association with the normal. There is a low nonprotein nitrogen content in the blood, and obesity is prevalent.

Type *G* is an important type, occurring practically exclusively in the female. It develops in certain girls at puberty. Once established it does not disappear at menopause. Hypertension, myocarditis, obesity and osteo-arthritis prevail. Endocrine disturbances are common.

Type *H*, the suggestive Cheyne-Stokes type, occurs in childhood but also develops from type *A* with which it is closely related. It is the inevitable precursor of type *I*. A high uric acid content of the blood and myocarditis occur in this type. Its associations with types *A*, *B*, *D*, *G* and *I* are numerous.

Type *I* is the typical Cheyne-Stokes type and develops from type *H* under certain conditions, among which is a high uric acid content in the blood. This type is seldom seen in a woman. It rarely occurs before the age of 50. While the patients in the majority of the cases in this type have cardiovascular renal disease or diabetes, there are some who develop it without suffering with either of the diseases mentioned; however, they all have an increase in uric acid.

At this point the question of necessity arises whether the respiratory types on the whole are caused by these abnormal physical states. As practically all disease processes occur in the presence of a normal type, serious doubt is cast on this point. It occurred to us that this question might be solved by studying respiratory types in children, making use of our patients as early in life as they could be tested. This line of research proved extremely valuable because it was found that types *A*, *B*, *D*, *E*, *F* and *H* may all occur in childhood. In fact we are forced to conclude that the types named are probably evidence of hereditary characteristics—perhaps an hereditary condition of the respiratory center. If this is the case, the preponderance of certain diseases in the types must be merely an associated condition.

To either confirm or deny the concept that the types are largely hereditary, a special study of this point was made. Three families were obtained in which the parents and at least four children could be tested. In each of two families five children were obtained; in the third, there were only four. A definite inheritance was shown.

CONCLUSIONS

1. All persons can be divided purely on the basis of their spirograms into about nine types which we have lettered. (A few extremely rare types are still to be studied.)

2. Respiratory types *A*, *B*, *D*, *E*, *F* and *H* are hereditary, and the occurrence of these types merely presages certain predispositions in the person.

3. Types *C* and *G* are controlled by some unusual function or influence of the female hormones.

4. Types *D* and *I* are similarly but to a more limited extent controlled by the male hormones.

5. Certain individual characteristics and disease processes are shown by this work to be of an hereditary nature. Namely, (1) cardiovascular renal disease; (2) nonulcerative colitis; (3) increased blood sugar, uric acid and nonprotein nitrogen content; (4) endocrine instability in general and specifically hyperthyroidism and hypothyroidism; (5) hypotension; (6) myocarditis; (7) nervous and mental states; (8) obesity and (9) osteo-arthritis.

6. As the hereditary predispositions to certain diseases can be determined in early childhood, the application of this work to preventive medicine is obvious.

ALBUMINURIA IN THE MECHANISM OF DETOXIFICATION *

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AND

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It is our purpose in this paper to present data indicating that the presence of serum proteins in the urine in nephritis is due to their combination with other nitrogenous fractions of tissue origin, some of which are highly toxic. It is our belief that this combination acts as a detoxicating mechanism and that the elimination of this protein by the kidneys is due to alteration of normal serum proteins by their combination with other split protein products which render them foreign to the circulation.

It has been apparent for some time that the renal element in nephritis is relatively unimportant and that theories of nephritis based solely on renal damage must be abandoned. The Aldrich and McClure [1] intradermal salt test as well as the series of experimental dehydrations reported by one of us [2] gives ample evidence that Martin Fischer was right in ascribing edema to a greater colloid affinity of the tissues for water, and that anuria may result from this factor alone. Experimental renal damage and partial or total nephrectomy do not produce the clinical picture of uremia, and clinically such cases are frequently reported with normal nonprotein nitrogen values in the blood. Similarly enormous high nitrogen levels have been found in the blood without uremic symptoms.

In a previous communication [3] this theory was elaborated, and the complete picture of uremia was reproduced by simply disturbing the mineral salt balance. Profound changes in the calcium-potassium ratio were reported, and it was suggested that under uremic conditions the

* Submitted for publication, Aug. 18, 1928.

* From the Department of Surgery and Physiological Chemistry of the University of Illinois School of Medicine.

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2. Andrews, Edmund: Water Metabolism: I., Arch. Int. Med. **37**:82 (Jan.) 1926; Water Metabolism: II., ibid. **37**:559 (April) 1926.

3. Andrews, Edmund: Experimental Uremia, Arch. Int. Med. **40**:548 (Oct.) 1927.

tissue permeability was increased to such an extent that products which were normally intracellular leaked into the blood. Later, tissue proteins were isolated from the urine in the early stages of both experimental and human nephritides, these proteins being recognized by precipitin reactions.[4] It is well known that most foreign proteins such as egg albumin or foreign serums injected parenterally will be excreted by the kidneys. Further work[5] confirmed our views that if tissue protein (liver) makes its way into the blood stream it will also pass through the kidneys just as a foreign protein will. A liver extract (containing, of course, both serum and liver proteins) injected intravenously into a normal animal will result in the passage of protein in the urine which is in a high degree specific to antiliver serums and does not react, even undiluted, with antiblood serums.

Pharmacologic studies of pure liver protein prepared by this means have shown that it is practically nontoxic and, therefore, is not the cause of clinical symptoms in nephritis. Former work has demonstrated unquestionably that the lower nitrogenous products are not the exciting cause of uremic symptoms, and it was predicted that substances of the proteose-peptone group would be identified as the offending agents.

This concept of nephritis, however, has signally failed to explain the mechanism of albuminuria. The proteins of the urine in nephritis are largely blood serum proteins, and the great stumbling block to the abandonment of older ideas of renal pathologic changes being fundamental was the question, why do apparently normal blood proteins pass through the kidney?

EXPERIMENTS

Studies of urinary proteins by precipitin reactions have at times yielded hitherto inexplicable results. In the usual process of purifying urinary proteins, they were saturated with ammonium sulphate up to the point where precipitation began (somewhere between 25 and 50 per cent saturation). The material is then set aside for crystallization. The first crop of crystals reacted in high dilution with antiblood serums. The second crop gave a much lower precipitin titer, and later crops failed almost entirely to react with antiblood serums. However, if the last crop which was not active with antiblood serums was injected into the rabbits, it produced an antiblood albumin blood serum of great strength. These experiments were tentatively interpreted as indicating that some substances had been in combination with the albumin which was broken off in the process of crystallization.

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4. Andrews, Edmund, and Thomas, W. A.: The Origin of Urinary Proteins, J. A. M. A. **90**:539 (Feb. 18) 1928.

5. MacDonald, R. F.; Thomas, W. A., and Andrews, Edmund: Proc. Soc. Exper. Biol. & Med. **25**:246, 1928.

The next step was the recognition by methods of dialysis recently published [6] that there was a change in the character of the serum protein in nephritis. Small amounts of serum were placed in tubes, the end of which was closed with collodion membrane and placed in hundredth normal hydrochloric acid. Nephritic serums took on large amounts of water, the normal serums practically none at all. Careful chemical studies of these serums have ruled out chlorides, nitrogenous production of the urea group, acidosis and other factors, except the proteins themselves, as being the cause of this phenomenon. Although the nephritic serums contained much less protein than normal serums, their hydrophilic properties were far greater.

The last two groups of experiments suggested that there was some change in the proteins in the blood and the urine, and that the urinary proteins were not simply unchanged blood proteins. With these points in view, we studied the mother liquor in the crystallization experiments already described and found that it contained large amounts of peptone. As much as 4 Gm. of peptone was recovered from a single batch of 18 liters of urine. A large number of further experiments yielded similar results. Again, if a solution of the precipitate of 50 per cent saturation with ammonium sulphate is dialyzed, peptone may be recognized in the dialysate. In the concentration of the dialysates, care must be taken to stop short of dryness in order to avoid increasing the coloring matter which will obscure results in both the biuret and the xanthoproteic tests.

This peptone was injected into dogs in varying amounts and was shown to be nontoxic. Two grams injected daily failed to produce any symptoms. In order to bring about even a transient albuminuria, a dose of 5 Gm. intravenously was needed. Six grams was the lethal dose. This fraction, then, did not represent the toxic element, and further studies of dialysates were made. It was found that these dialysates contained a highly toxic protein. This protein is coagulable by heat. It reacts strongly with antiblood albumin and antieuglobulin serums. It does not react with antifibrinogen serums. However, its state of dispersion has been so changed, possibly by combination with some toxic factor, that it will readily pass through a collodion membrane. There is another possibility to be considered, i. e., that the protein itself is toxic simply by virtue of its greater dispersion. Intravenous injection of 50 mg. of this protein into dogs will produce deep coma and death in from twenty-four to forty-eight hours. In the early stages of the coma, the dog will twitch violently if touched. Later, the coma is so deep that he cannot be aroused by any means.

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6. Thomas, W. A., and Andrews, Edmund: A New Clinical Test for Tissue Thirst, Proc. Soc. Exper. Biol. & Med. **25**:773 (June) 1928.

Similar results may be obtained with uremic serums. The dialysate of such serums contains a small amount of protein of the peptone proteose group; in our experiments, about 100 cc. of uremic serum has been necessary to provide enough proteins to give recognizable reactions in the concentrated dialysate. The dialysis is carried on for only twelve hours, as in longer periods there is a possibility of hydrolysis of the serum protein. Control experiments on normal serums fail to show this phenomena. The amount of dialyzable protein recovered from human serums has thus far been too small to identify.

Urinary proteins of the dog have been studied; it was found that after crystallization and prolonged dialysis, those proteins did not produce toxic symptoms when injected intravenously into dogs, but if the same urinary proteins are subjected to dialysis for only a few hours to rid them of the more readily fusible urinary substances, they are toxic and will produce convulsions, coma and albuminuria, if injected intravenously.

COMMENT

From the foregoing data, the following hypothesis is offered as a tentative suggestion of the mechanism in uremia and nephritis.

Changes in the osmotic equilibrium are brought about. These are associated with alterations in the mineral salt balance which produce a markedly increased tissue permeability. This allows leakage into the blood stream, first, of normal tissue proteins, which are ordinarily retained within cell membranes. As these proteins are foreign to the circulation, they are excreted by the kidneys. Later, there follows a leakage into the circulation of other split protein products, certain of which are highly toxic and produce the symptoms of uremia. These substances are combined with the proteins of the blood or absorbed by them. This protein complex seems to have a smaller molecular aggregate or to be in a higher state of dispersion so that not only will it pass through the kidneys but part of it will even pass through a collodion membrane. The peptone itself is at least not free because if the serum protein is precipitated by heat or by saturation by ammonium sulphate, the peptone is precipitated with it, in crystallization or to a small extent by dialysis; it is freed from the larger albumin molecule and may then be identified; the protein bearing the toxic substances or which is toxic by reason of its higher state of dispersion passes readily through a collodion membrane, and if dialysis is continued for four or five days, the water being changed daily, subsequent dialysis yields a dialysate that reacts negatively to the biuret test.

Albuminuria may then be looked on as a protective mechanism on the part of the body to carry poisons from the seat of origin to the outside by the well known mechanism of neutralization by combination with

innocuous substances. These substances may represent normal stages in nitrogen metabolism, and the pathologic factor lies in their making their way into the blood stream, as already suggested. Another interesting possibility to be considered is that nephritis is comparable to diabetes in the sense that protein metabolism is interfered with by some means, so that oxidation is incomplete and the process stops at a state where the products of nitrogenous metabolism are toxic and are eliminated by the mechanism previously described.

CONCLUSION

1. The urine of nephritic persons contains, besides the normal blood proteins, a relatively nontoxic peptone combined or adsorbed by serum proteins and also a highly toxic blood protein so highly dispersed in solution as to pass readily through a collodion membrane.

2. The hypothesis is presented that albuminuria is a detoxicating mechanism for eliminating poisonous products of protein metabolism.

Book Reviews

NURSES, PATIENTS AND POCKETBOOKS. A Report of the Study of the Economics of Nursing, Conducted by the Committee on the Grading of Nursing Schools. By MAY AYRES BURGESS, Directress. Price, $2. 1928.

This is a report on a nation-wide study of supply and demand in nursing service. It is in a sense a preliminary report in a projected five years' study of the nursing situation by a committee of twenty-one members, fourteen of whom are officially appointed by the national organizations and seven elected as members-at-large. This is a fact-finding committee organized for the purpose of grading training schools.

The first part of the volume is devoted to information on the economics of nursing; the second part to an analysis of this information.

It was the original intent of the committee to first take up the grading of training schools, but it was later decided to study the supply and demand for nurses. This study reveals a marked oversupply in the face of a rapidly increasing number of graduates. They estimate that 17,500 graduated in 1926, compared with 3,456 in 1900. The training schools during this period increased from 432 to 2,155. During this time the number of nurses per hundred thousand of population has increased from 16 to 141, while the number of physicians has decreased from 173 to 137.

Registers, especially from large cities, indicate that the supply is equal to the demand with prospects of an oversupply in the immediate future. Public health organizations have more applicants than positions.

In addition to the topics mentioned, many other subjects are studied in detail, an effort being made to supply statistical data. This book furnishes valuable information to all those interested in nursing. When the work is completed and further volumes are published, a splendid reference work on the nursing problem will be available.

A TEXTBOOK OF ACTINOTHERAPY. By D. D. ROSENWARNE, M.R.C.S., (England), L.R.C.P. (London). Price, $4. Pp. 237, with 20 illustrations, including a colored plate. St. Louis: C. V. Mosby Company, 1928.

The first sixty-two pages are taken up with fundamental physical and electrical facts, such as are given in a course of high school physics. The biologic action of light is discussed on pages from 73 to 103. Pages from 113 to 226 are devoted to clinical procedures, with a great deal of emphasis on autointoxication and colonic flushing. On pages from 143 to 176 diseases of the skin are dealt with, largely from the standpoint of light therapy.

The text is verbose, arrangement is often illogical and undue repetition is apparent. It contains many glaring errors, such as: "Seborrhoeic Eczema—Affection of the sudoriferous glands," and "Sycosis, Eczema of the Hair Follicles." In the discussion of psoriasis, the author states: "X-rays combined with ultra-violet radiations are probably the best treatment. . . ."

One gathers that cutaneous hyperpigmentation following actinotherapy is produced by the short light waves acting on nerve plexuses and affecting the sympathetic nerves. This evidently acts on the suprarenal glands, as later: "The production of pigments by the adrenals under the influence of light." On the contrary, it has been shown repeatedly that the oxidase of the pigment-producing cell is increased. There is no method for demonstrating the melanogen.

The author complicates the application of actinotherapy, probably more so than is indicated by the present day knowledge.

The text contains some valuable material, but numerous errors tend to destroy one's confidence, and reduce its value.

The illustrations are excellent, and a bibliography is appended.

A TEXT-BOOK OF INFECTIOUS DISEASES. By GOODALL and WASHBOURN. Revised and in large part rewritten by E. W. GOODALL, O.B.E., M.D., B.S. Third edition. Price, $10. Pp. 709, with 26 plates, 9 other photographs, 15 diagrams and 34 charts. New York: William Wood & Company, 1928.

In bringing up-to-date his "Manual," the author has expanded it into a good sized textbook. He has added an introductory chapter on epidemiology which should prove interesting and stimulating to all students of infectious diseases. It fails, however, to cover the field as adequately as even such a brief and general discussion may reasonably be expected to do. The more recent work of Webster on experimental epidemiology may be cited as an example of a pertinent omission.

The clinical symptoms and signs of the various diseases are well described; the methods of treatment are up-to-date. But the presentation of the facts and theories concerning etiology leaves something to be desired. The problem of etiology is, in the case of several diseases, admittedly a vexatious one, and the author in his preface warns the reader that he is not a bacteriologist. Nevertheless, he cannot be unconditionally pardoned for his failure to mention, for instance, the studies of Dochez on scarlet fever, or those of the several workers who believe that measles is caused by a filterable virus.

The book is well printed and bound and contains numerous photographs, diagrams and fever charts, all of which should be highly instructive to students and practitioners.

SPECIAL CYTOLOGY. Edited by EDMUND V. COWDRY. Two Volumes. Price, $20. Pp. 1,300. New York: Paul B. Hoeber, Inc.

The two volumes comprising this work are made up of thirty-five sections by as many authors, covering the various special cells of the body. Each of the sections is largely monographic in character, since the writers have all made extensive contributions to the subjects with which they are dealing. The result is a work of the highest value which is unique in nature. Here within the scope of 1,300 pages is contained all the most important knowledge of the structure and function of the body cells. The introductory chapter indicates the new directions which research in cell function will probably take and mentions some of the possibilities of the more newly developed methods of study in this field. The different systems of cells, the skin and its derivatives, the respiratory tract, digestive tract, etc., are then taken up in an interesting and scholarly way. In addition to the anatomy and functional activity of the individual or group of cells, the organization of the cells into specific tissues as well as the embryologic and comparative anatomic aspects are discussed. In certain sections the general reactions of cells to disease agents and the changes occurring in pathologic states are touched on, but the subject matter does not include pathologic histology as might perhaps be inferred from the subtitle. This not only is a textbook for students of biology and medicine but is also an invaluable reference work for those engaged in special problems of cell biology. The book is beautifully printed. The illustrations are abundant and well executed, and each section is followed by an extensive bibliography.

CLINICAL STUDIES ON INFECTIONS OF THE URINARY PASSAGES IN CHILDREN (DANISH). By DR. ARNE JOHANNESSEN. Pp. 350. Copenhagen: Levin & Munksgaard, 1926.

This is also a thesis for the M.D. degree done in the Pediatrics Department of the University of Copenhagen, under the direction of Dr. Bloch. The second half of the monograph contains brief case records and histories of more than 100 patients investigated. The monograph is interesting as an illustration of the type of consecutive work and grasp of the literature in one field required for the M.D. degree in Denmark. Otherwise the study cannot be said to advance particularly the problem under investigation. The author concludes (on page 137)

that hematogenous origin of cystitis is most prevalent during the first year of life and that after the second year the entrance of bacteria through the external passages is assuming a more important rôle.

SYSTEMIC INFECTIONS. THEIR DIAGNOSIS AND TREATMENT. By A. KNYVETT GORDON, M.B., B.C., B.A. Cantab., Medical Superintendent of the Virol Pathological Research Laboratories, Consulting Pathologist to the Great Western Railway Medical Fund Society and to the Victoria Hospital, Swindon. Price, $4. New York: William Wood & Company, 1928.

According to the preface this book is intended primarily for those engaged in general practice. But, in the opinion of the reviewer, the book can be read with profit only by the most poorly trained physician or by one who has completely forgotten the technic of thorough examination of patients and of the commonest laboratory procedures. Chapters are devoted to elementary bacteriology and hematology, and a large portion of the book to the methods of searching for a focus of infection and of the treatment thereof.

PSYCHONEUROSEN FUNKTIONELLE NEUROSEN ERSCHÖPFUNGSZUSTÄNDE. VON DR. WALDEMAR UNGER. Praktische Differentialdiagnostik. Band II, Neurologie. Teil 2. Price, 8 marks. Mit 3 Abbildungen. Dresden und Leipzig: Verlag von Theodor Steinkopff, 1927.

The book is of special advantage to the general practitioner facing the problems of diagnosis and therapy of functional disturbances of the psyche. The author surveys in a systematic manner the clinical pictures of exhaustions, constitutional disturbances, organic neuroses, hysteria, compulsion-neuroses, psychopathic personalities, migraine, epilepsy and combined pictures. He then describes precisely diagnosis according to the most important clinical features. The value of this study lies in general in the attempt of the author to do justice to the variety of syndromes, not forgetting the individual in favor of a preconceived idea of a psychopathologic unit.

KLINISKE STUDIEN OVER URINVEJSINFEKTIONER HOS BØRN. By ARNE JOHANNESSEN. Pp. 350. Copenhagen: Levin & Munksgaard, 1926.

This manuscript is a thesis for a doctor's degree. There are 100 complete case histories of pyelitis included, with excellent references, but no new ideas are advanced as to treatment. The Norgaard pyometer is used to determine the amount of pus in the urine, as this method is more accurate than the microscopic estimation.

BIDRAG TIL STUDIET AF ASTHMA SAERLIG HOS BØRN. By KAJ BOAGOE. Pp. 284. Copenhagen: Levin & Munksgaard, 1926.

This is a thesis for a doctor's degree. It contains 232 instructive case histories with excellent references. There are not, however, any new ideas expressed on the subject.

CONTRIBUTION TO THE STUDY OF ASTHMA ESPECIALLY IN CHILDREN (DANISH). By DR. KAJ BAAGOE. Pp. 284. Copenhagen: Levin & Munksgaard, 1926.

This is a clinical study done under the direction of Dr. Bloch, and the monograph is presented as a thesis for the M.D. degree. The work embraced a study of between 200 and 300 patients. The literature is presented and discussed ably and critically, but there is nothing essentially new in either the facts or the theory presented.

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